FORMULATION AND EVALUATION OF DIACEREIN LOADED MICROSPONGES IN CAPSULE

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI – 600 032

in partial fulfilment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

submitted by

Register Number:261411267

under the guidance of

Prof. K. Elango, M.Pharm., (Ph.D),

Professor and Head

Department of Pharmaceutics

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

APRIL – 2016

DEPARTMENT OF PHARMACEUTICS
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003
TAMILNADU

DATE:

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF DIACEREIN LOADED MICROSPONGES IN CAPSULE"** submitted by the candidate with **Register No. 261411267** to The Tamil Nadu Dr.M.G.R. Medical University is evaluated.

1.

2.

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003
TAMILNADU

CERTIFICATE

This is to certify that the dissertation entitled **FORMULATION AND EVALUATION OF DIACEREIN LOADED MICROSPONGES IN CAPSULE"** submitted by the candidate with **Register No. 261411267** in partial fulfillment of the requirements for award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICS** by The Tamil Nadu Dr. M.G.R. Medical University is a Bonafide work done by her during the academic year 2015-2016.

Place: Chennai – 03

Date:

(Dr. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A)

DEPARTMENT OF PHARMACEUTICS
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003
TAMILNADU

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF DIACEREIN LOADED MICROSPONGES IN CAPSULE"** submitted by the candidate with **Register No. 261411267** in partial fulfilment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICS** by The Tamil Nadu Dr. M.G.R. Medical University is a bonafide work done by her during the academic year 2015-2016 under my guidance.

Place: Chennai – 03

Date:

[Prof. K.ELANGO, M.Pharm., (Ph.D.),]

ACKNOWLEDGEMENT

There are many who have contributed in one way or another to complete my studies and my thesis. I am indebted to them for their direct support and collaboration with me.

First of all I would like to thank **The Almighty** for showering his blessings on me, giving me great strength, health, endurance, courage and determination to complete my studies and my thesis.

I am deeply thankful to my Principal **Dr. A. Jerad Suresh**, M.Pharm., Ph.D., M.B.A, College of Pharmacy, Madras Medical College, Chennai-03.

I am tremendously grateful to my guide **Prof. K. Elango** M.Pharm.,(Ph.D.), Professor and Head , Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai-03 for his continuous guidance, supportive suggestions, innovative ideas which made me to do my thesis the best.

I express my whole hearted thankfulness to the Staff members, Mr**. K. Ramesh Kumar** M.Pharm., Mr**. N. Deattu** M.Pharm., Ph.D, Mrs. **S. Daisy Chellakumari** M.Pharm., Ph.D, Mrs. R**. Devi Damayanthi** M.Pharm., Ph.D, Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai-03 for their immense support and encouragement for completing my thesis.

A special word of thanks goes to all non-teaching staff members Mr. **Marthandam**, Mrs. **Shankar**i, Mrs**. Raziya**, Mrs**. Rama** Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai-03, Mr. **Sivakumar**, Department of Pharmaceutical Chemistry.

It gives me immense pleasure in thanking all my dear friends **Deepa Joseph**, D. **Immaneul Nesakumar, C. Kanchana, M. Meenakshi, D. Mohana Priya, T. Nandhini, D. Sai Dharshini, C. Saranya, V. Sundhar Rajan** for continuous encouragement, suggestions and technical assistance.

I extend cordial thanks to all my **Seniors** and **Juniors** for their help and guidance till the end.

The **members of my family** are quietly impressed and have good understanding on me and led me to a determination to continue learning and I had hoped that would happen, while knowing there was no real ways to influence that outcome.

CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

API	Active Pharmaceutical Ingredient
BCD	Beta Cyclodextrin
BCS	Biopharmaceutical Classification System
CT	Computed Tomography
DCN	Diacerein
DMSO	Dimethyl Sulphoxide
DSC	Differential Scanning Colorimetry
EC	Ethyl Cellulose
EUD	Eudragit RS 100
F. Code	Formulation Code
FT-IR	Fourier Transform Infra Red
GIT	Gastro Intestinal Tract
HPLC	High Pressure Liquid Chromatography
HPMC	Hydroxy Propyl Methyl Cellulose
HPTLC	High Pressure Thin Layer Chromatography
ICH	International Conference on Hormonization
MDDS	Microsponge Drug Delivery System
MS	Magnesium Stearate
NC	No Change
NDDS	Novel Drug Delivery System
NMR	Nuclear Magnetic Resonance
NSAID	Non Steroidal Anti Inflammatory Disease
OA	Osteoarthritis
PEG	Poly Ethylene Glycol
PHEMA	Polyhydroxyethyl methacrylate

PVA	Poly Vinyl Alcohol
PVP	Poly Vinyl Pyrrolidine
RH	Relative Humidity
RP-HPLC	Reverse Phase High Pressure Liquid Chromagraphy
SCMC	Sodium Carboxy Methyl Cellulose
SD	Standard Deviation
SEM	Scanning Electron Microscopy
TDS	Transdermal Delivery System
TEC	Triethyl Citrate
UV	Ultraviolet
XRPD	X- Ray Powder Diffraction
rpm	Revolutions per minute
mg	Milligram
g	Gram
ml	Milliliter
µm	Micrometer
%	Percentage
% w/w	Percentage weight by weight
min	Minute
mm	Millimeter
Hrs	Hours
µg	Microgram
nm	Nanometer

THE NOVEL DRUG DELIVERY[1]

The aim of the Novel drug delivery system is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug delivery system should deliver drug in rate controlled manner in the body over a specific term of treatment.

This idealized objective switches to the two main aspects they are as follows

I. **Spatial drug delivery**

Targeting a drug to a particular organ or tissue.

II.**Temporal drug delivery**

Drug delivery rate to target tissue is controlled.

The prime areas of research and development for NDDS are

1. Liposomes
2. Niosomes
3. Nanoparticles
4. Transdermal drug delivery
5. Implants
6. Oral system
7. Microencapsulation/ Microcapsules

Novel drug delivery system can be divided into 2 systems

a. Sustained release drug delivery system

b. Controlled release drug delivery system

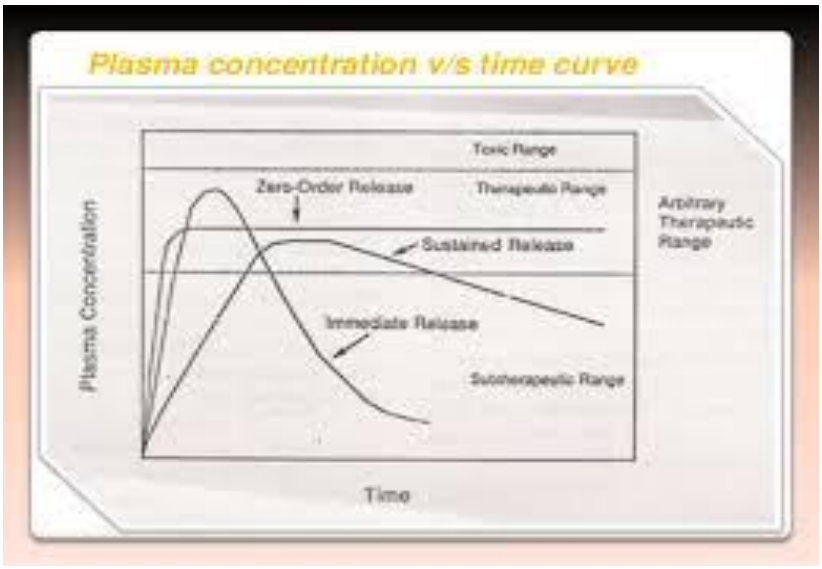


Fig No.1.1 Plasma concentration Vs. Time curve

Sustained release drug delivery system

It is a Pharmaceutical dosage form to retard the release of a therapeutic effect such that it looks in the systematic circulation is delayed and the plasma profile is sustained in duration. The onset of its Pharmaceutical action is often slow and the duration of its therapeutic effect is sustained.

Controlled release drug delivery system

This system has a meaning that goes beyond the scope of sustained drug action it manifests a predictability and reproducibility in the drug release kinetics. The release of drug from a controlled release drug delivery system gains at a rate profile that is not only predictable kinetically but also reproduced from one unit to another.

I. Rate pre-programmed drug delivery system
II. Activation- modulated drug delivery system
III. Feed – back regulated drug delivery system
IV. Site- targeting drug delivery system

Merits of Controlled drug delivery system[1]

- Improved patient convenience and compliance.
- Reduction in fluctuation in steady state levels.

- Increased safety margin of high potency drugs due to better control of plasma levels.
- Maximum utilization of drug enabling reduction in total amount of dose administered.
- Reduced in health care cost through
    - ❖ Improved therapy.
    - ❖ Shorter treatment period.
    - ❖ Lower frequency of dosing.
    - ❖ Reduction in personnel time to dispense, administer and monitor patients.

Demerits of CDDS[1]

1. Decreased systemic availability in comparison to immediate release conventional dosage forms, which may be due to

i. Incomplete release

ii. Increased first-pass metabolism

iii. Increased instability

iv. Insufficient residence time for complete release

v. Site specific absorption

v. pH dependent stability etc.

2. Poor *in-vitro – in- vivo* correlation.

3. Possibility of dose dumping due to food, physiologic or formulation variables or chewing or grinding of oral formulations by the patient and thus, increased risk of toxicity.

4. Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.

5. Reduced potential for dosage adjustment of drugs normally administered in varying strengths.

ORAL CONTROLLED DRUG DELIVERY SYSTEM[2]

Oral route has been the commonly adopted and most convenient route for the drug delivery. Oral route of administration has been received more attention in the pharmaceutical field because of the more flexibility in the designing of dosage form than drug delivery design for the other routes.

In last two decades the drug delivery technology has been developed rapidly and many novel oral drug delivery systems have been invented. Despite tablets, capsules, suspension, emulsion and solution, they are more superior to the conventional oral formulation. Because of their clinical advantages over immediate release pharmaceutical products containing the same drugs, sustained release system and controlled release drug delivery systems are developed which are interchangeable.

Some oral controlled drug delivery formulations

- Osmotic tablets
- Mucoadhesive tablets
- Matrix tablets
- Film coated tablets
- Enteric coated tablets
- Swellable tablets
- Floating capsules
- Microgranules and spheroids
- Beads
- Pellets
- Microcapsules and microspheres

DISCOVERY OF MICROSPONGE DRUG DELIVERY[3]

In the current years the development of new drugs is not sufficient for the drug treatment. But it also involves the development of suitable drug delivery system at site of action. The *in-vivo* fate of the drug is not only determined by the properties of the drug, but it is also determined by the carrier system, which permits a controlled and localized release of the active drug according to the specific need of the therapy. The

biggest challenge up to date is to control the delivery rate of the medicaments by various modern technologies met by extensive research.

However, TDS is not practical for delivery of materials whose final target is the skin itself. The controlled release of drug from the formulation into the epidermis such that the drug remains primarily localized with only a restricted amount entering the systemic circulation, is a means of controlling side-effects. Thus, the need exists for delivery systems to maximize the period of time that an active ingredient is present, either on the skin surface or within the epidermis while minimizing its transdermal penetration into the body. Another potential problem in topical delivery of drugs relates to uncontrolled evaporation of the active ingredient, unpleasant odour, the use of unaesthetic vehicles which may be greasy, sticky and may cause discolorations, since this can result in the lack of patient compliance.

Carrier technology is the potential solution to these challenges. Microparticles and nanoparticles have been increasingly researched to achieve targeted and sustained release of drugs. These include microspheres, liposomes, and nanoparticles etc. which alter the absorption and release characteristics of the drug. Microspheres are unable to control the release rate of drug from itself. Once the outer wall is ruptured the drug contained within microspheres will be released from it. Liposomes having demerits like lower drug entrapment, difficulty in preparing formulation, limited chemical stability and microbial stability so the preservatives are required. Solid lipid nanoparticles are having most of the benefits in the topical drug delivery. Nanomaterial can easily enter in to the systemic circulation by inhalation or ingestion, and possibly also via skin absorption, especially if the skin is damaged. Once in the blood stream, nanomaterials can be transported around the body and are taken up by organs and tissues including the brain, heart, liver, kidneys, spleen, bone marrow and nervous system.

The microsponge-based polymeric microspheres uniquely overcome problems associate with above technologies. Microsponges are extremely small, inert, indestructible spheres that do not pass through the skin. Rather, they collect in the tiny nooks and crannies of the skin and slowly release the entrapped drug, as the skin needs it. They are designed to deliver a pharmaceutical active ingredient efficiently at

the minimum dose and also to enhance stability, reduce side effects and modify drug release.

Microsponges are microscopic spheres capable of absorbing skin secretions, therefore reducing oiliness and shine from the skin. Spherical particles composed of clusters of even tinier spheres are capable of holding four times their weight in skin secretions. These products are typically presented to the consumer in conventional forms like creams, gels or lotions and they contain a relatively high concentration of active ingredients. Recently their use is also being investigated for oral drug delivery. This article provides concise information to the various aspects of the structure, development, applications and future of microsponges. It is to be introductory to the vast amount of research that has been done and the large number of opportunities that exist in the field of microsponges.

MICROSPONGE DRUG DELIVERY SYSTEM

In recent years, there has been considerable emphasis given to develop novel Microsponge based drug delivery systems, in order to modify and control the release behaviour of the drugs. By incorporation into a carrier system, it is possible to alter the therapeutic index and duration of the activity of drugs.

The microsponge technology was developed by Won in 1987 and the original patents were assigned to Advanced Polymer Systems, Inc. Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge-like spherical particles with a large porous surface. They may enhance stability, reduce side effects and modify drug release favourably. Microsponge technology has many favourable characteristics, which make it a versatile drug delivery vehicle.The Scanning Electron Microscopy of the microsponge particle reveals that its internal structure as the "bag of marbles". The porosity is due to the interstitial spaces between the marbles. The interstitial pores can entrap many wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens, anti-infective and anti-inflammatory agents. These entrapped microsponges may then integrated or formulated into product forms, such as creams, lotions, powders, soaps, capsules and tablets.[4,5]

Although the microsponge size may vary, a typical 25 μm sphere can have up to 250000 pores and an internal pore structure equivalent to 10 ft in length, providing a total pore volume of about 1 ml/g. This results in a large reservoir within each microsponge, which can be loaded with up to its own weight of the active agent. The microsponge particles themselves are too large to be absorbed into the skin and this adds a measure of safety to these microsponge materials. Another safety concern is the potential bacterial contamination of the materials entrapped in the microsponge. As the size of the pore diameter is smaller, the bacteria ranging from 0.007 to 0.2 μm cannot penetrate into the tunnel structure of the microsponges.[4]

Fig No.1.2 A Microsponge

Potential features of microsponge drug delivery system[6,7]

1. Stable over a pH range of 1 to 11.
2. Stable up to 130^{o}C temperature.
3. Compatible with the many of the vehicles and active ingredients.
4. Self sterilizing as their average pore size is 0.25µm where bacteria cannot penetrate.
5. Higher pay load (50 to 60%).
6. Free flowing and cost effective.
7. Reveal good compatibility with various vehicles and ingredients.
8. Can absorb oil up to 6 times their weight without drying.
9. Characterized by free flowing properties.

Benefits of Microsponge Drug Delivery System[5,8]

- Enhanced product performance.
- Extended release.
- Reduced irritation and hence improved patient Compliance.
- Improved product elegancy.
- Improved formulation flexibility.
- Improved thermal, physical, and chemical stability.
- Flexibility to develop novel product forms.
- Non-irritating, non-mutagenic, non-allergenic and non-toxic.
- Allows incorporation of immiscible substances.

Advantages of microsponges over other technologies and delivery systems

Microencapsulation, liposomes and ointments [10]

1. Microsponges offer better control of drug release than microcapsules. Microcapsules cannot usually control the release rate of the API. Once the wall is ruptured, the API contained within the microcapsules will be released.
2. Microsponges show better chemical stability, higher payload and easier formulation compared with liposomes.
3. In contrast to ointments, microsponges have the ability to absorb skin secretions, therefore, reducing greasiness and shine from the skin. Ointments are often aesthetically unappealing, greasy and sticky, resulting in lack of patient compliance.

Topical drug delivery system [9]

Conventional formulations of topical drugs are intended to work on the outer layers of the skin. Such products release their active ingredients upon application, producing a highly concentrated layer of active ingredient that is rapidly absorbed. When compared to conventional formulation, the Microsponge system can prevent excessive accumulation of ingredients within the epidermis and the dermis. Potentially, the Microsponge system can reduce significantly the irritation of effective drugs without reducing their efficacy. Microsponges are extremely small, inert, indestructible spheres that do not pass through the skin. Rather, they collect in the tiny nooks and crannies of the skin and slowly release the entrapped drug, as the skin needs it.

Oral drug delivery [10]

1. Preserve the active ingredients within a protected environment and offer oral controlled delivery to the lower part of the GIT.

2. Microsponge systems improve the solubility of poorly soluble drugs by entrapping these drugs in their porous structure.

3. As the porous structure of the microsponge is very small in size, the drugs entrapped will be reduced to microscopic particles with higher surface area, and consequently improved rate of solubilisation.

4. Maximize the amount of drugs to be absorbed, as the time it takes the microsponge system to pass through the intestine is considerably increased.

Properties of the actives for the entrapment into the microsponge [3]

i. It should be either fully miscible in monomer or capable of being made miscible by addition of small amount of a water immiscible solvent.
ii. It should be water immiscible or at most only slightly soluble.
iii. It should be inert to monomers and should not increase the viscosity of the mixture during formulation.
iv. It should be stable when in contact with polymerization catalyst and under conditions of polymerization.
v. The spherical structure of the microsponges should not collapse.

Formulation [3,4,9]

The MDS contain drug, polymer, vehicle and other additives like plasticizers that help to stabilize the structure.

Various drugs used in MDS : Benzoyl peroxide, Mupirocine, Tretinoin, Aceclofenac, Flucinolone, acetonide, Ketoprofen, Paracetamol , Dicyclomine, Fluconazol, Hydroquinone etc.

Polymers used: Ethyl cellulose, Eudragit RS 100, Polystyrene, acrylic polymers and PHEMA etc as they can form a microsponge "cage".

Vehicles used: Dichloromethane, acetone, ethanol.

Plasticizers: Triethylcitrate, dibutyl phthlate.

Preparation of Microsponges [4,11]

Drug loading in microsponges drug delivery system is done in two ways, one step process or by two step process as discussed in liquid-liquid suspension polymerization and quasi emulsion solvent diffusion techniques which are based on physicochemical properties of drug to be loaded. If the drug is typically an inert non-polar material, will create the porous structure it is called porogen. Porogen drug,

which neither hinders the polymerization nor become activated by it and stable to free radicals is entrapped with one step process.

Liquid-Liquid Suspension Polymerization

The porous microspheres are prepared by suspension polymerization method in liquid-liquid systems . In their preparation, the monomers are first dissolved along with active ingredients in a suitable solvent solution of monomer and then dispersed in the aqueous phase, which consist of additives (surfactant, suspending agents, etc.). The polymerization is then initiated by adding catalyst or by increasing temperature or irradiation .

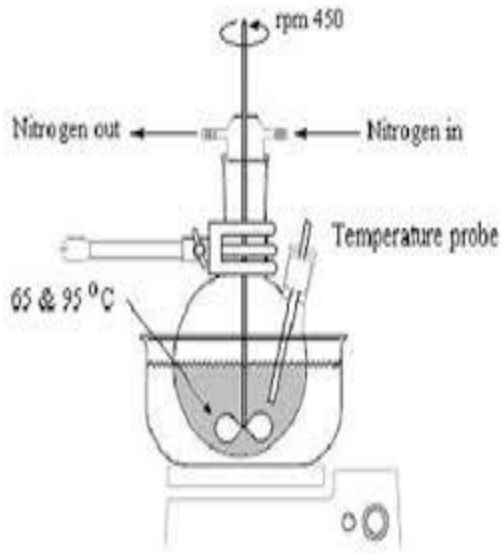
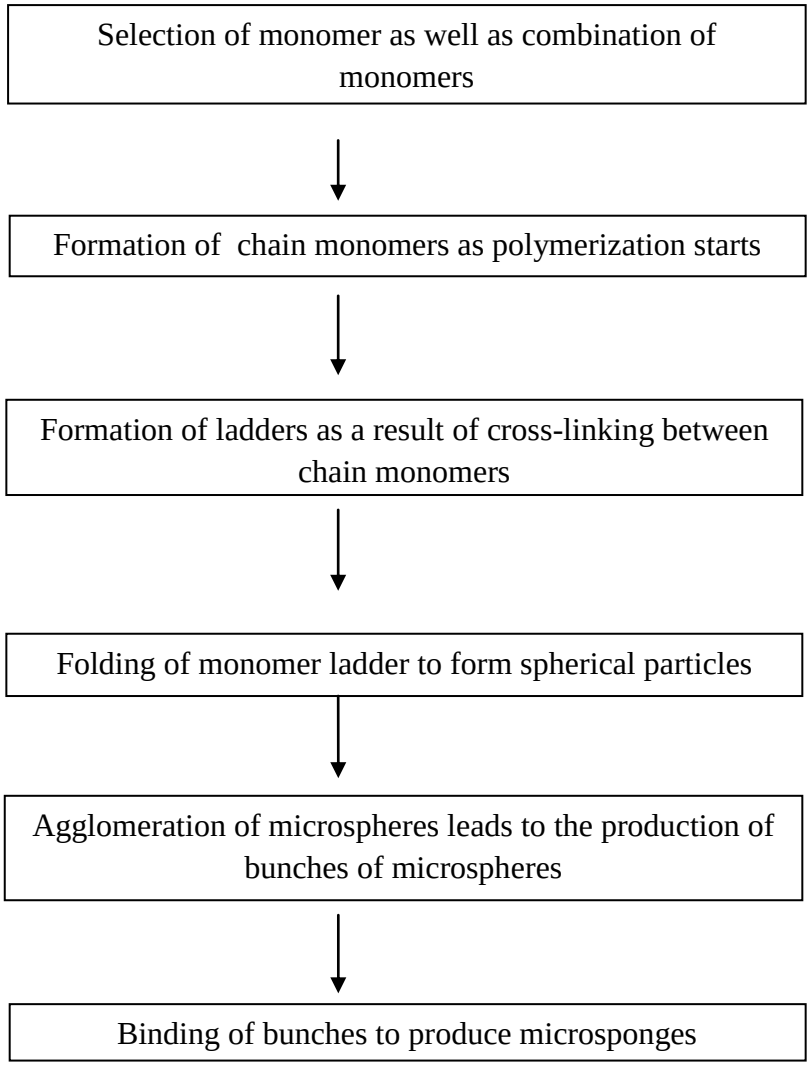


Fig No.:1.3 REACTION VESSEL FOR MICROSPONGE PREPARATION BY LIQUID-LIQUID SUSPENSION METHOD

The polymerization process continues the formation of a reservoir type of system with spherical structure. After the polymerization process the solvent is removed leaving the spherical structured porous microspheres, i.e., microsponges. The various steps involved in the preparation of microsponges are summarized below:

STEPS IN THE PREPARATION OF MICROSPONGES:



Quasi-emulsion Solvent Diffusion

All microsponges were prepared by a quasi-emulsion solvent diffusion method using an external phase containing distilled water and PVA. The internal phase consisted of Drug, Solvent, polymer and TEC, which was added at an amount of 20% of the polymer in order to facilitate the plasticity. At first, the internal phase was prepared and added to the external phase at room temperature. After emulsification, the mixture was continuously stirred for 2 hours. Then the mixture was filtered to separate the microsponges. The product was washed and dried by hot air oven at 40°C for 12 hours .

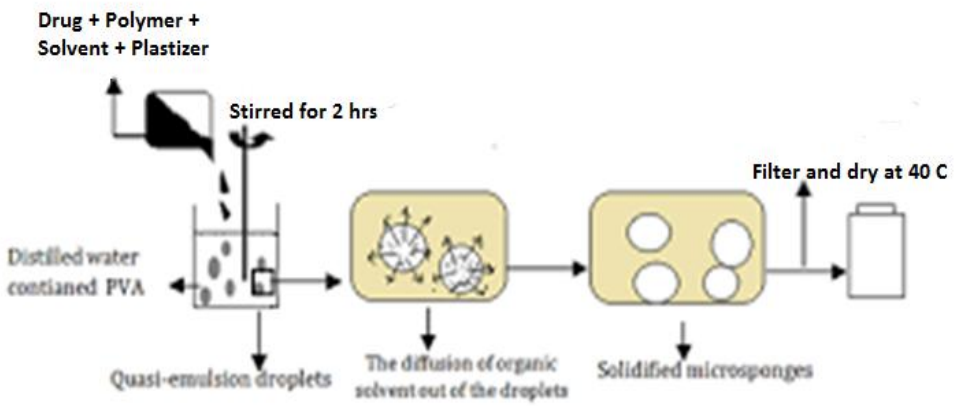


Fig No.1.4 PREPARATION OF MICROSPONGES BY QUASI-EMULSION SOLVENT DIFFUSION METHOD.

Limitations:[7]

Both the methods usually use organic solvents as porogens, which pose an environmental hazard, as some may be highly inflammable, posing a safety hazard. Moreover, in case of the Bottom-Up approach traces of residual monomers have been observed, which may be toxic and hazardous to health. While the limitations seem to be serious, they can be easily overcome, by using proper quality control measures and proper washing post manufacture coupled with good standardization of the various processes .

Drug release mechanisms [3]

The mentioned programmable parameters can be effectively manipulated to design Microsponge delivery system for the release of functional substance over a period of time in response to one or more external stimuli. The release mechanism of this system is mainly:-

A. Sustained or Timed Release

In the development of a sustained release Microsponge, different physical and chemical parameters of the entrapped active substance such as volatility, viscosity and solubility will be studied while in case of polymeric microsponge pore diameter, volume, and resiliency of the polymeric microsponge are evaluated to give necessary sustained release effects.

B. Release on Command

Microsponges can be designed to release the given amounts of active ingredients over time in response to one or more external triggers.

1. Pressure Release

Microsponge system releases fluid or active ingredient when it is pressed or squeezed, thereby replenishing the level of entrapped active ingredient onto the skin. The amount released may also depend upon the release of the sponge and the resiliency of the Microsponges.

2. Temperature Release

The release of active ingredients from microsponges can be activated by temperature. At room temperature, few entrapped active ingredients can be too viscous to flow suddenly from microsponges onto the skin. With increase in skin temperature, flow rate is also increased and therefore release is also enhanced.

3. pH

Triggering the pH-based release of the active can be achieved by modifying the coating on the microsponge. This has many applications in drug delivery.

4. Solubility

Microsponges loaded with water miscible ingredients like antiseptics and antiperspirants will release the ingredient in the presence of water. The release can also be activated by diffusion but taking into consideration, the partition coefficient of the ingredient between the microsponges and the external system.

Safety consideration[9]

Safety studies of microsponges can be established by:

- Eye irritation studies in rabbits.
- Skin irritation studies in rabbits.
- Mutagenicity in bacteria.
- Oral toxicity studies in rats.
- Allergenicity in guinea pigs.

Applications of microsponges[12]

Few applications of microsponge drug delivery system are as follows:

- Microsponges in Oral Care Cosmetics

An interesting application of the microsponge technology could be in oral cosmetics, such as to sustain the release of volatile ingredients, thus increasing the duration of the 'fresh feel'. Microsponges of such volatile ingredients may be easily incorporated in tooth pastes or mouth washes.

- Long lasting Coloured Cosmetics:A new application for Microsponges

Colours entrapped in microsponges may be used in a variety of coloured cosmetic products such as rouge or lipsticks to make them long lasting. As stated above, microsponges help in uniform spreading and improving covering power. Thus, colored cosmetics formulated with microsponges would be highly elegant.

- Going the natural way using a Functional Active

Although natural actives are important consumer attractants, now the focus has shifted on using multifunctional natural ingredients. For example, Marinosomes®, liposomes made from natural antiinflammatory lipid extracts, have set a new paradigm in using such functional 'active excipients'. The possibility of using such substances for constructing a microsponge structure appears to be cost effective and innovative.

Table No.1.1: Applications along with advantages of MDDS[4]

S.No.	Application	Advantages
1.	**Anti-acne**: Eg: Benzoyl peroxide	Maintained efficacy with decreased skin irritation and sensitization.
2.	**Anti-inflammatory**: Eg. Hydrocortisone	Long lasting activity with reduction of skin allergic response and dermatoses.
3.	**Sunscreens**:	Long lasting product efficacy with improved protection against sunburns and sun related injuries even at elevated concentration and with reduced irritancy and sensitization.
4.	**Anti-fungals**:	Sustained release of actives ingredient.
5.	**Anti-dandruffs**:Eg. Zinc pyrithione, selenium sulphide.	Reduced unpleasant odour with lowered irritation with extended safety and efficacy.
6.	**Antipruritics**:	Extended and improved activity.
7.	**Skin depigmenting**: Eg: Hydroquinone	Improved stabilization against oxidation with improved efficacy and aesthetic agents appeal.
8.	**Rubefacients**:	Prolonged activity with reduced irritancy greasiness and odour.

Table No.:1.2 Examples of microsponge drug delivery with their formulations[4]

MDS	Drug	Disease
Gels	Benzoyl peroxide	Anti- acne treatment
	Diclofenac sodium	Inflammation
	Terbinafine HCl	Anti-fungal
	Hydroxyzine HCl	Urticaria and atopic dermatitis
	Mupirocin	Antibacterical activity
Creams	Hydroquinone and retinol	Melanoma
Implants	Poly(DL- lactic-co-glycolic acid)	Sin tissue engineering
Lotions	Benzoyl peroxide	Anti-acne treatment
Tablets	Ketoprofen	Musculoskeletal pain
	Paracetamol	Colon targeting
	Meloxicam	Arthritis
Others	Benzoyl peroxide	Anti- acne treatment
	Ibuprofen	NSAID

Table No.:1.3 Marketed formulations of microsponges[13]

Product name	Advantages	Company
Carac cream, 0.5%	Carac cream contains 0.5% fluoruracil with 0.35% being incorporated into a patented porous microsphere(Microsponge) composed of methyl methacrylate cross polymer and dimethicone	Dermik laboratories, Inc. Berwyn,PA 19312 USA
Retinol cream	The retinol molecule is kept in the microsponge system to protect te potency of vitamin A. This helps to maximize the retinol dosage, wthile reducing the possibility of irritation. Retinol is a topical vitamin A derivative, which helps maintain healthy skin, hair and mucous membranes.	Biomedic
Retin- A-Micro	0.1 and 0.04% tretinoin entraped in MDS, for topical treatment of acne vulgaris. This formulation uses patented methyl methcrylated cross polymer porous microspheres	Ortho-Mc Neil Pharmaceutical, Inc.
EpinQuin micro	The Microsponge system entrap hydroquinone and retinol. The microsponges release these ingredients into the skin gradually throughout the day, which may minimize skin irritation.	Skin Medica Inc.
Oil control lotion	Feature light lotion microsponges that absorb oil on the skin's surface during the day, for a matte finish. Eliminate shine for hours with this feature- weight lotion.	Fountain cosmetics

Sports cream RS and XS	Topical analgesic, anti-inflammatory and counter irritant actives in a MDS for the management of musculosketal conditions.	Embil Pharmaceutical Co. Ltd.
Line Eliminator dual retinol facial treatment	Light weight cream with a retinol in MDS, delivers both immediate and time released wrinkle fighting action.	Avon
Retinol 15 night cream	A night time treatment with microsponge technology using a stabilize formula of pure Retinol and vitamin A.	Sothys
Salicylic peel 20	Deep BHA peeling agent for salicyclic acid 20% microsponge technology. Excellent exfoliations and stimulation of skin for more resistant skin types or for faster results.	Biophora
Salicylic peel 30	Deep BHA peeling agent for salicyclic acid 30% microsponge technology. Excellent exfoliations and stimulation of skin for more resistant skin types or for faster results.	Biomedic

Table No.1.4: Patents filed related to microsponges[9]

Patent No.	Inventors	Publications Date
US4690825	Won. Richard	1987
US4863856	Dean RC Jr et al.	1989
US5292512	Schaefer et al	1989
US5135740	Katz et al	1992
US5679374	Fanchon; Chantal et al	1994
US5316774	Eury, Robert P et al	1994
US5725869	Lo; Ray J.R	1996
US6395300	Straub et al	1999
US6211250	Tomlinson et al	2001
US20030232091	Shefer et al	2005
US20040247632	Cattaneo, M	2004
US20050271702	Wright,Steven G et al	2005

Recent advances in microsponge drug delivery [9]

Various advances were made by modifying the methods to form nanosponges, nanoferrosponges and porous microbeads.

β-CD nanosponges were also developed that can be used for hydrophobic as well as hydrophilic drugs, in contrast to polymeric micro or nanosponges. These advanced systems were studied for oral administration of dexamethasone, flurbiprofen, doxorubicin hydrochloride, itraconazole and serum albumin as model drug. These nanosponges were developed by cross-linking the β-CD molecule by re-acting the β-CD with diphenyl carbonate. Some researchers also observed the nanosponges as good carrier for the delivery of gases. Researchers also observed that incorporating a cytotoxic in a nanosponge carrier system can increase the potency of the drug suggesting that these carriers can be potentially used for targeting the cancerous cells.

Nanoferrosponge, a novel approach constituted the self-performing carriers having better penetration to the targeted site due to the external magnetic trigger

which enforces the carriers to penetrate to the deeper tissue and then causing the removal of magnetic material from the particle leaving a porous system.

Due to the improved characteristics of porous microspheres, process was developed to produce the porous micro beads. This method (High Internal Phase Emulsion, HIPE) consisted of the monomer containing continuous oil phase, cross linking agent and aqueous internal phase. They also observed an improved stability of RNA and the relatively effective encapsulation process of siRNA. The approach could lead to novel therapeutic routes for siRNA delivery.

Future prospects [3]

Microsponge drug delivery system holds a promising opportunity in various pharmaceutical applications in the upcoming future as it has unique properties like enhanced product performance and elegancy, extended release, improved drug release profile, reduced irritation, improved physical, chemical and thermal stability which makes it flexible to develop novel product forms. The real challenge in future:

- The development of the delivery system for the oral peptide delivery by varying ratio of polymers.
- The use of bioerodible and biodegradable polymers for the drug delivery is enabling it for the safe delivery of the active material.
- As these porous systems have also been studied for the drug delivery through pulmonary route which shows that these system can show effective drug release even in the scarce of the dissolution fluid thus colon is an effective site for targeting for drug release.
- These carriers also require to be developed for alternative drug administration routes like parenteral and pulmonary route.
- These particles can also be used as the cell culture media and thus can also be employed for stem cell culture and cellular regeneration in the body.
- Due to their elegance, these carrier systems have also found their application in cosmetics.

These developments enabled researchers to utilize them variably. These novelties in formulation also open new ways for drug deliver.

Literature related to MDS

1. **Vikas Jain *et al.*[16]** formulated Paracetamol loaded Eudragit based microsponges using quasi-emulsion solvent diffusion method. Shape and surface morphology of the microsponges were examined using SEM. The formulations were subjected to *in-vitro* release studies and the results were evaluated kinetically and statistically. The *in-vitro* release data showed a biphasic pattern with an initial burst effect. The release kinetics showed that the data followed higuchi model and the main mechanism of drug release was diffusion.

2. **Vikas Jain *et al.*[17]** developed a prolonged MDS system containing Dicyclomine. Dicyclomine loaded Eudragit based microsponges were prepared using quasi-emulsion solvent diffusion method. Increase in drug: polymer ratio resulted in a reduction in the release rate of the drug from the microsponges. Kinetic analysis showed that the main mechanism of drug release was by higuchi matrix-controlled diffusion. The unique compressibility of microsponges can be applied to achieve effective local action.

3. **Anil kumar *et al.*[18]** prepared Fenoprofen microsponges by Quasi-emulsion solvent diffusion method. Four different drug-polymer ratio was prepared and performed experimental design by changing concentration of solvent. Prepared microsponges were formulated as tablets and compressed using chitosin: HPMC mixture for colon targeting. Optimised microsponge formulations were subjected to *in-vitro* dissolution studies and found to modify the release rate. The colon specific tablets release the drug at 8^{th} hour due to addition of ß- glycosidase and continued upto 18^{th} hour.

4. **Riyaz Ali *et al.*[19]** developed Domperidone for augmented gastroparesis therapy using quasi emulsion solvent diffusion method using Eudragit RS 100 with various drug- polymer ratios. Developed systems was more proficient to give extended dug release, superior in contrast to conventional marketed formulation. Characterization techniques were done and microsponges were

loaded in capsules followed by *in-vitro* drug release study.1:2 drug-polymer ratio showed extended release when compared to conventional marketed formulation Domstal.

5. **Karthika *et al.*[20]** prepared microsponges containing Lornoxicam and Eudragit RS 100 were by quasi emulsion solvent diffusion method. The effects of drug to polymer ratios on physical characteristics of the microsponges were investigated. Compatibility of drug with adjuncts was studied by FT-IR. Production yield, loading efficiency, particle size analysis, surface morphology and *in-vitro* release studies were carried out. The microsponges were compressed into tablets. The effects of different stirring rates, amount of solvent, amount of emulsifier used on the physical characteristics of the microsponges were investigated. *In-vitro* dissolution studies were done on all formulations and the results were kinetically evaluated and the release rate of Lornoxicam was found to be modified.

6. **Sonali *et al.*[21]** developed Prednisolone loaded microsponges for colon specific drug delivery by using quasi-emulsion solvent diffusion method with Eudragit RS 100 as polymer. Evaluation like FTIR, SEM, particle size, production yield, drug entrapment efficiency were examined. *In-vitro* dissolution studies showed modified release for 8 hours and best fitted a zero order kinetic model. Plasma drug concentration of drug was also studied for optimized formulation and C_{max}, t_{max} and AUC was also observed.

7. **Rashmi *et al.*[22]** formulated curcumin microsponges for colon specific drug delivery by Quasi-emulsion solvent diffusion method using 3^2 full factorial design. Prepared microsponges were optimized in order to analyze the effects of independent variables (volume of ethanol and Eudragit L100) on the encapsulation efficiency ,particle size, and drug release. The optimized formulation was subjected to *in-vivo* study using acetic acid induced colitis model in rats. Release studies revealed that microsponges prevented the premature release of curcumin in upper GIT and specifically released the drug at colonic pH.

8. **Ramani Gade *et al.*[23]** developed Hydroxyzine hydrochloride microsponges using polymer methocel 10000 cps and in combination with Eudragit –S 100, Eudragit-L 100, Eudragit-RL 100 and Eudragit-RS 100. These are prepared by oil in oil emulsion solvent diffusion method using acetone as dispersing solvent and liquid paraffin as the continuous medium. Compatibility of the drug with adjuncts was studied by FT-IR. Production yield, loading efficiency, particle size analysis, surface morphology and *in-vitro* release studies were carried out. The microsponge tablet formulation, F11 showed controlled release of hydroxyzine hydrochloride for 12hrs.

9. **Shah Harsh *et al.*[24]** prepared Aceclofenac microsponges using polymers like Ethyl cellulose, Eudragit RS 100, Eudragit S100 and Eudragit RL 100 in different concentrations. Evaluations were performed and optimized batch was formulated as colon targeting tablet. *In- vitro* dissolution study showed controlled release upto 24 hours. This technique have enhanced solubility, flow properties and compression characteristics.

10. **Riyaz Ali *et al.*[25]** formulated and evaluated gel containing microsponges of Diclofenac diethylamine by using eudragit RS 100 with varied drug- polymer ratios. They were characterized by SEM,DSC, FTIR, XRPD and particle size analysis and evaluated for morphology, drug loading *in-vitro* drug release and *ex- vivo* diffusion as well.

11. **John I D'souza *et al.*[26]** formulated Fluocinolone Aceonide microporous microparticles to control the release of drug to the skin. Microsponges were prepared by quasi emulsion solvent diffusion method. Production yield, loading efficiency, particle size analysis and surface morphology were performed. Surface morphology by SEM revealed microporous nature of microsponges. Drug release was observed controlled with comparative anti-inflammatory active with the gels containing free drug.

12. **Markand Mehta *et al.*[27]** prepared Clotrimazole microsponge by emulsion solvent diffusion technique by using Ethyl cellulose , HPMC K4M, Carbopol 934, Eudragit RS 100, Eudragit S 100, Eudragit RL 100 and evaluated for % practical yield, % loading efficiency and *in- vitro* drug release study. The drug release data of optimized batch were fitted into different kinetic models which show that the drug release from gel formulations follows zero order release. So encapsulation of clotrimazole into microsponge would modify the release rate and also reduce side effects.

13. **Hamid Hussain *et al.*[28]** developed gel loaded microsponges of diclofenac sodium by quasi emulsion technique employing ethyl cellulose as polymer. Compatibility of drug-excipients was done by FTIR. SEM, particle size, production yield, drug entrapment efficiency were examined. Prepared microsponge was dispersed in carbopol gel base for controlled delivery to the skin. Spreadility, pH determination, *in-vitro* diffusion study using KC cell were performed.

14. **Makwana Rajshree *et al.*[29]** carried Photostability enhancement of Miconazole nitrate by ethyl cellulose microsponges formulation using Quasi-emulsion technique. The prepared microsponges were subjected to *in-vitro* dissolution studies, FTIR and DSC. Microsponge showed higher photostability as compared to plain drug and other physical mixture.

15. **Ramadevi *et al.*[30]** formulated Itraconazole loaded microsponges using Eudragit RS 100 by quasi emulsion solvent diffusion method. The formulations were evaluated for % loading efficiency, drug entrapment efficiency, morphology, surface topography by SEM, *in-vitro* release, release kinetics, pore diameter were measured. Release rate was found to be controlled for 6 hours.

16. **Mahajan Aniruddha G *et al.*[31]** prepared controlled release formulation of Indomethacin microsponges by quasi emulsion solvent diffusion method. Microsponges were evaluated by micromeritic properties, drug content,

encapsulation efficiency and particle size. *In- vitro* dissolution study indicated that the release of Indomethacin varied according to the concentration of matrix forming polymer.

17. **Ravi *et al.*[32]** prepared Erythromycin microsponges using quasi emulsion solvent diffusion method. Erythromycin microsponges were then incorporated into a Carbopol-940 gel prepared by hydrogel technique for release studies. The best formulation was found to be stable at room temperature for 3 months. Thus it was concluded that erythromycin can be formulated as microsponge gel that can release the drug upto 8hrs with reduced side effects.

18. **Mohana Kumar *et al.*[33]** prepared Mupirocin microsponges using an emulsion solvent diffusion method. FT-IR and SEM was used to study the shape and morphology of microsponges. Mupirocin microsponges were then incorporated into a vanishing cream base for release studies. It was shown that the drug: polymer ratio, stirring rate, volume of external and internal phase influenced the particle size and drug release behavior of microsponges. The results showed that an increase in the ratio of the drug: polymer resulted in a reduction in the release rate of Mupirocin from microsponges. Kinetic analysis showed that the main mechanism of drug release was by Higuchi matrix-controlled diffusion.

19. **Swetha A *et al.*[34]** formulated microsponges containing Ethylcellulose and Eudragit RS 100 by quasi emulsion solvent diffusion method using Etodolac. The effects of different drug to polymer ratios on physical characteristics of microsponges were investigated. Surface morphology, particle size and pore structure of the microsponges were examined. *In- vitro* dissolution study showed that the release rate of the drug has been modified.

20. **P.Yadav *et al.*[35]** prepared Microsponge loaded controlled release formulations of Acyclovir quasi emulsion solvent diffusion method. They were characterized for particle size, production yield and entrapment efficiency. The range of production yield was found between 57.43% and 77.81%,

entrapment efficiency was found between 68.95 % and 87.56% and particle size was found between 376.3 µm to 777.7 µm for different batches. Porous structure of microsponges was confirmed by SEM. Best optimized batch was incorporated in carbopol and aloe gel and lipstick base. *In- vitro* release studies using diffusion cell revealed that the drug release followed Korsemeyer Peppas model.

21. **Atmaram *et al.*[36]** formulated oxybenzone microsponges by quasi emulsion solvent diffusion method. The effects of Ethyl cellulose and Dichloromethane were optimized by the 32 factorial design. The optimized microsponges were dispersed into the hydrogel and further evaluated. It showed enchanced sun protection factor compared to the marketed preparation with reduced irritation and toxicity.

22 **Chainesh *et al.*[37]** prepared Fluconazole Microsponges by quasi emulsion solvent diffusion method using Ethyl cellulose as a polymer, polyvinyl alcohol, Acetone as Internal phase volume and Liquid paraffin as External phase volume. Optimization of Fluconazole loaded Microsponge was done by 24 Factorial Designs. The optimized batch of Fluconazole and Aceclofenac loaded Microsponge was evaluated by particle size, surface morphology and topography by SEM, Drug-Excipients compatibility study using FTIR. The *in-vitro* drug release from the microsponges hrs was found to be extended up to 10 Hrs.

23. **Jaya Raj Kumar *et al.*[38]** prepared ketotifen microsponges with three different proportions of ethyl cellulose by quasi-emulsion solvent diffusion method. These formulations were studied for particle size and physical characterization. These microsponges enriched gel formulation were prepared by using 2 and 3 % w/w of SCMC and studied for viscosity, pH, gel strength, spreadability, bioadhesive force, drug content, *in-vitro* release, HPLC and SEM analysis. The optimized formulations were able to release the drug up to 8 hours.

24. **Roaa *et al.*[39]** developed microsponges gel of poorly soluble drug meloxicam microsponges by quasi-emulsion solvent diffusion method. The effects of

drug:polymer ratio, stirring time and Eudragit polymer type on the physical characteristics of microsponges were investigated and characterized for production yield, loading efficiency, particle size, surface morphology, and *in-vitro* drug release from microsponges showed that the microsponge with Eudragit L100 polymer had optimum physical properties and enhanced the dissolution and release when compared with other formulae and pure drug.

25. **Ahamed Abbas Hussain *et al.*[40]** prepared Ketoconazole microsponges by quasi emulsion solvent technique using different types of Eudragits as Eudragit E 100, Eudragit RS or Eudragit RL. Physicochemical interaction between drug and excipients as individual one, physical mixture and prepared microsponge has been evaluated using FTIR and DSC.

26. **Kirti *et al.*[41]** prepared Diclofenac Sodium loaded Eudragit microsponges using quasi emulsion solvent diffusion method. Different drug: polymer ratios were used to formulate the microsponges. The compatibility of the drug with polymer was established. Surface morphology of the microsponges was examined using SEM. Production yield, loading efficiency, particle size analysis, and *in-vitro* release studies were carried out. *In-vitro* release study showed that the release of drug was in controlled manner and it was increased with increase in drug to polymer ratio up to certain limit.

27. **Ravi *et al.*[42]** prepared Erythromycin microsponges using quasi emulsion solvent diffusion method. The SEM and DSC studies were carried out to study shape, morphology of microsponges and thermal analysis respectively Thus it was concluded that erythromycin can be formulated as microsponge gel that can release the drug up to 8hrs with reduced side effects.

Literature related to DCN

28. **Prashant S. Walke *et al.*[43]** enchanced solubility of DCN by Mannitol solid dispersions and they were prepared in ratio 1:1, 1:3 and 1:5 by physical triturating, solvent evaporation and fusion method. The results showed marked increase in the saturation solubility and dissolution rate of DCN as compared to pure drug. A mannitol dispersion (1:5) prepared by fusion method showed

excellent physiochemical characteristics and was described by dissolution kinetics. It was the best formulation in this study.

29. **Nitin Maski *et al.*[44]** studied solubility of ßCD – DCN inclusion complex. Phase solubility profile indicated that the solubility of DCN was significantly increased in presence of β-cyclodextrin and it was classified as AL- type, indicating the 1:1 stoichiometric inclusion complexes. The complexes formed were quite stable. The solid complexes prepared by physical mixture, co-evaporation/solid dispersion, kneading method and precipitation method was characterized using Differential Scanning Calorimetry, Powder X-ray Diffractometry and FTIR. *In-vitro* study showed that the solubility and dissolution rate of DCN was significantly improved by complexation with β-cyclodextrin.

30. **Patel Krushika *et al.*[45]** studied the formulation and evaluation of Buccal tablet of the poorly soluble drug DCN. It was prepared by direct compression using combination of bioadhesive polymers like Carbopol 934P, HPMC , Sodium alginate, SCMC, HPC, PVP K 30 in different ratio. The swelling index of tablet increased with increasing amount of Carbopol 934P. Ethyl cellulose was found as optimum formulation.

31. **Randa *et al.*[46]** formulated and evaluated DCN loaded niosomes in order to improve dissolution hence its oral bioavailability. The formulations were prepared by thin film hydration. Method using different ratios of cholesterol to non ionic surfactants (Span 20 and 60) 1:2 and 1:3. The order of encapsulation efficiency niosomes indicates better release profile compared to free DCN. It was concluded that it is possible to enhance solubility and ultimately improve bioavailability of the drug by this promising approach.

32. **Janki Patel *et al.*[47]** developed a emulgel for the topical delivery of the poorly water soluble drug DCN. The influence of the concentration of gelling agent Carbopol 940(0.75%, 1%,1.25%) the concentration of both the emulsifying agent (2.5%,1% w/w of mixture of Span 20 and Tween 20) and the oil phase

(6%,7%,8% w/w of Liquid paraffin) . The results of *in-vitro* drug release showed that carbopol 940(1%) based emulgel gave better release.

33. **Deshmukh *et al.*[48]** studied the improvement in solubility and dissolution rate of a poorly soluble drug. DCN by solid dispersion method using PVP K30 and HPMC E4 as carriers. Four different formulations were prepared by solvent evaporation method using varying drug: carrier ratios 1:1,1:2,1:3,1:4. Formulation containing drug: polymer ratio of 1:4 with PVP K30 showed the best release as compared to the Pure drug. It was concluded that PVP K30 as a carrier can be well utilized to improve the solubility of poorly soluble drug.

34. **Kotta Kranthi Kumar *et al.*[49]** studied the development of formulation and evaluation of DCN cream which are designed to enhance the onset of action. The cream is designed by two phase system. The cream is formed by using the fusion technique. The percentage of drug content, pH, color,viscosity, spreadability and extrudability shown per stability study after three months, it gives the accurate and satisfactory results.

35. **Randa *et al.*[50]** improved solubility and dissolution rate of DCN by solid dispersion method. Binary solid dispersions were made using PEG-4000 or PEG-6000 as carriers with varying drug: carrier ratios 1:1, 1:3 and 1:5. Also ternary solid dispersions were made using PEG-4000 and Pluronic F-68 at ratios 1:5:1, 1:5:2 and 1:5:3. Formula SD9 (1:5:3; drug: PEG-4000: Pluronic F-68) showed the best dissolution profile with about 44.73% of the drug being released in the first 5 minutes and more than 79 % of the drug being released in the first 15 minutes. It was concluded that combination of PEG-4000 and Pluronic F-68 can be well utilized.

36. **Richa *et al.*[51]** developed press coated tablet of DCN and Chlorzoxazone for arthritis. Drug-polymer compatibility studies were carried out by FT-IR. Core tablet was prepared by direct compression using Super disintegrant Sodium Starch Glycolate. The core tablet was compression coated with different quantities of coating material containing different polymers. The press coated

tablets coated with HPMC K4M:HPMC K100 in 64.39:35.61 ratios with 200 mg coat weight are most likely to provide the desired delivery of DCN and Chlorzoxazone.

37. **Subramania Nainar *et al*[52]**. investigated the use of HPMC polymer to formulated sustained release matrix tablets containing DCN. Prepared by wet granulation method. The release of drug showed non-fickian diffusion obeying first order kinetics.

38. **Dhanashri Subash Yadav *et al.*[53]** developed novel microcrystallization technique in an attempt to enhance dissolution behaviour. Acetone and water were used as solvent and antisolvent system respectively while PVPK30, PEG 6000 and PXMR 188 were used as polymers in crystallization process. The microcrystals were characterized by XRPD SEM,FTIR and dissolution test. It was found that dissolution characteristics of microcrystals were significantly improved than that of pure DCN. Also XRPD, FTIR reflected altered molecular arrangement in their structure.

39. **Veren wally *et al.*[54]** hypothesized that a topical formulation of DCN 1% DCN cream for one armpit and placebo for the other. The number of blisters was reduced significantly below the initial level even during withdrawal in four patients.

Literature related to analysis of DCN

40. **Chitlange *et al.*[55]** described a stability-indicating HPTLC method for analysis of DCN in bulk and pharmaceutical dosage form. Precoated silica gel 60 F254 plate was used as stationary phase. The separation was carried out using Toluene: Isopropyl alcohol: Ammonia (4.6:4.6:0.8 %v/v/v) as mobile phase. The Rf value for the drug was found to be 0.30±0.01. The linearity was obtained in the range 100-350ng/band (r2 = 0.9909). The method was validated as per ICH guidelines. DCN was subjected to forced degradation by

acid, alkali, oxidation and dry heat. The degradation products were well resolved from the pure drug with significantly different Rf values.

41. **Sanjay *et al***. [56] developed two simple and sensitive colorimetric methods for quantitative estimation of Diacerin from Pharmaceutical Capsule dosage form. Developed methods are based on the formation of Chloroform extractable ion pair coloured complex of drug with Orange II (Method A) and Tropoelin (Method B). The complex formed in method A and B showed maximum absorbance at 435. Linearity was obeyed in concentration range of 50-350 µgm/ml and 50250 µgm/ml of Diacerein for method A and B respectively. The results of analysis were validated statistically and by recovery studies.

42. **Pulla reddy *et al*.** [57] performed chromatographic analysis on Agilent, Zebra C18 reversed phase column with mobile phase consisting Ammonium acetate: acetonitrile in the ratio 60:40% v/v, at a flow rate of 1.0 ml/min and eluents monitored at 267nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per International Conference on Harmonization (ICH) guidelines.

43. **Appala raju *et al*.** [58] developed reverse phase HPLC method for the estimation of DCN in tablet dosage form. An Inertsil ODS-3V analytical column (250 x 4.6 mm, 5 µm partical size) with mobile phase consisting of mixture of buffer 0.03M Potassium Dihydrogen Orthophosphate in water and pH adjusted to 3.20 with Ortho-phosphoric acid and Acetonitrile in the gradient program was used. The method was validated by determining its accuracy, precision and linearity. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of DCN in bulk drug and in its pharmaceutical tablet dosage form.

44. **Selvam *et al*.**[59] developed a novel, simple, rapid and sensitive spectrophotometric method for the estimation of DCN in capsule dosage form. In this method Acetonitrile was used as solvent. The maximum absorption was

found to be 255nm. Beer's law was obeyed in the concentration range of 2-10µg/mL. The method is found to be rapid, precise and accurate and can be employed in the laboratory for the routine estimation of the drug.

45. **Vijayageetha *et a.*[60]** developed simultaneous spectrophotometric determination of DCN and Aceclofenac tablets by partial least squares and principal component regression methods do not require any prior graphical treatment of the overlapping spectra of two drugs in the mixture. Chemometric methods were found satisfactorily for determination.

46. **Narendra kumar *et al.*[61]** developed a simple, rapid and accurate colorimetric method for the estimation of DCN in bulk and pharmaceutical dosage forms, method are based on pink colored complex formation between DCN with sodium hydroxide which shows maximum absorbance at 514 nm. The linearity was found to be 10-50µg/ml. The developed method was found to be precise and accurate form the statistical validation of the analysis.

47. **Khemchand *et al.*[62]** developed for the quantitative estimation of DCN from Pharmaceutical Capsule dosage form based on the solubility of DCN in Phosphate buffer having pH 6.8. The drug showed maximum absorbance at 258 nm. Linearity was obeyed in concentration range of 5-30 µg/ml. The results of analysis were validated statistically and by recovery studies.

48. **Gurupaddaya *et al.*[63]** developed a spectrophotometric method for determination of DCN in pure form and pharmaceutical formulations based on the reaction of carboxylic acid group of the drug with a mixture of potassium iodate (KIO3) and potassium iodide (KI) in aqueous medium at room temperature. The reaction followed yellow colored chromogen spectrophotometrically measured absorbance at 352 nm. Beer's law was obeyed in the concentration range 2-12 µg/ml with molar absorptivity and Sandell's sensitivity of 2.8339 x 104 L mol/cm and 0.001360 µg/cm2 per 0.001 absorbance units, respectively

49. **Dharamveer *et al.*[64]** developed for simultaneous estimation of DCN and Aceclofenac from tablet dosage form. 0.1M acidic methanol was used as solvent. First method, Simultaneous equation method, involves the measurement of absorbances at two wavelengths 256.0 nm (max of DCN) and 276.0 nm (max of Aceclofenac), Second method is First order derivative spectroscopy, wavelength selected for quantitation were 250.0 nm for DCN (zero cross for Aceclofenac) and 256.8 nm for Aceclofenac (zero cross for DCN) and third method is Area under curve method, area under curve in the range of 251.0-261.0 nm (for DCN) and 271.0281.0 nm (for Aceclofenac) were selected for the analysis. The proposed methods were found to be rapid, specific, precise, accurate and can be successfully applied for the routine analysis of DCN and Aceclofenac in bulk and combined dosage form

Aim and plan of work

AIM OF WORK

1. To formulate Diacerein microsponges using Eudragit RS 100 and Ethyl Cellulose polymers.

2. To study the effect of Concentration of External phase, amount of Internal phase and stirring rate on optimised formulation.

3. To enclose Diacerein microsponges in Hard gelatin Capsules.

The objective of current work is to enhance the rate of solubility of the drug thus to augment bioavailability and to release the drug in a controlled manner to improve the efficacy and patient compliance.

PLAN OF WORK

The present work was planned to carry out the Formulation and evaluation of Microsponges loaded with Diacerein as model drug.

The design of work:

I. Compatibility studies

- Physical compatibility study.
- Chemical compatibility study.

II. Preparation of Standard graph for Diacerein.

III. Formulation and development

- Preparation of Diacerein microsponges by Quasi emulsion solvent diffusion method.
- Evaluation of prepared microsponges.
    - ❖ Production yield.
    - ❖ Particle size determination.

- ❖ Drug content.
- ❖ Loading efficiency.
- ❖ Surface topography.

- Preformulation studies of optimized microsponge
- Filling of Capsules

IV. Evaluation of Capsules

- ✓ Uniformity of weight.
- ✓ Disintegration test.
- ✓ *In-vitro* release study of capsules.
- ✓ Drug Content

V. Evaluation of Optimised formulation

- ❖ Effect of concentration of External phase.
- ❖ Effect of Concentration of Internal phase.
- ❖ Effect of Stirring rate.

VI. *In-vitro* anti-inflammatory activity.

VII. Release kinetics of optimized formulation.

OSTEOARTHRITIS [65]

- Arthritis is a chronic degenerative disease that affects the joints of the body. Types include: Osteoarthritis, Rheumatoid arthritis, Psoriatic arthritis etc.
- Osteoarthritis (wear and tear arthritis) is the most common type of arthritis. It is associated with a breakdown of cartilage in joints and can occur in almost any joint in the body. It commonly occurs in the weight-bearing joints of the hips, knees, and spine. It also affects the fingers, thumb, neck, and large toe.
- Osteoarthritis usually does not affect other joints unless previous injury , excessive stress or an underlying disorder of cartilage is involved.
- It causes the cartilage in a joint to become stiff and lose its elasticity, making it more susceptible to damage. Over time, the cartilage may wear away in some areas, greatly decreasing its ability to act as a shock absorber. As the cartilage deteriorates, tendons and ligaments stretch, causing pain. If the condition worsens, the bones could rub against each other.

RATIONALE FOR THE DRUG [52, 78]

- NSAIDs are a class of drugs used to treat inflammation and pain but their use increases the risk of upper gastrointestinal adverse effects and does not affect the underlying pathogenesis of articular diseases thus have minimal role in modifying disease course and improving quality of life.
- DCN is a newly introduced Symptomatic drug for OA bone metabolism Pharmacological group. It is a semi- synthetic anthraquinone derivative extracted from certain plants.
- In contrast to NSAIDs, DCN does not inhibit synthesis of prostaglandins, hence no gastro duodenal toxicity has been observed. Therefore, a safer symptomatic drug for OA.
- DCN belongs to BCS class II (Low solubility and High permeability), thus selected to augment the bioavailability of the drug.
- DCN is characterized by rapid clearance due its shorter half life and thus warrants the use of sustained release formulation for prolonged action to

improve its patient compliance. Therefore it is preferable to develop a method to deliver the drug in a sustained manner.

RATIONALE OF DRUG DELIVERY SYSTEM [3, 39,20]

A set of reasons to select Microsponge drug delivery are as follows

1. It offers extended release upto 12 hours i.e A 25µm sphere can have a total pore length of about 10ft with a pore volume of about 1ml/g and can have up to 25,000 pores. This provides an extensive surface area for high entrapment. Because of entrapment and adsorption of actives onto the polymeric cage, the release of actives is prolonged.
2. It enhances the rate of dissolution of poorly soluble drugs by entrapping them in their pores. As these pores are very small the drug released is reduced to microscopic particles. Therefore, decrease in particle size by micronization of such drugs result in an increase in dissolution rate and so the bioavailability.
3. It is believed to contribute towards reduced side effects, better tolerance, improved stability, increased elegance along with formulation flexibility.
4. They are stable over a pH range of 1 to 11, temperature upto 130^{o} C. It also gives a higher load (50 to 60%) but still it is free flowing.
5. One of the best feature of MDS is its Self-sterilizing. As the size of the pore diameter is smaller, the bacteria ranging from 0.007 to 0.2µm cannot penetrate into the tunnel structure of the microsponges.

OSTEOARTHRITIS [65, 66]

Osteoarthritis (OA) represents failure of the diarthrodial (movable, synovial-lined) joint. It is also erroneously called as degenerative joint disease. OA is primarily a disease of cartilage that reflects a failure of the chondrocyte to maintain proper balance between cartilage formation and destruction. This leads to loss of cartilage in the joint, local inflammation, pathologic changes in underlying bone and further damage to cartilage triggered by the affected bone.

According to the American College of Rheumatology, OA may be of;

1. **Idiopathic**

   i. Localized (spine, knee, hip, hands, feet, elbow, shoulder and other joints)
   ii. Generalized (involving 3 or more joints)

2. **Secondary**

   i. Trauma
   ii. Developmental and congenital diseases (dyplasia etc)
   iii. Metabolic diseases (gout etc.)
   iv. Endocrine disorders (diabetes, hypothyroidism etc.)
   v. Calcium deposition diseases (pyrophosphate, hydroxyapatite etc.)
   vi. Other bone and joint diseases (rheumatoid arthritis etc.)
   vii. Neuropathic (Charcot) arthropathy
   viii. Septic arthritis

Epidemiology

OA is the most common joint disease of humans. Among the elderly, knee OA is the leading cause of chronic disability in developed countries. Some 100,000 people in the US are unable to walk independently from bed to bathroom because of OA of the knee or hip.

- In older individuals, hip OA is more common in men, while OA of interphalangeal joints and the thumb base is more common in women. Similarly symptomatic knee OA is more common in women than in men.

- Racial differences exist in both the prevalence of OA and the pattern of joint involvement. The Chinese in Hong Kong have a lower incidence of hip OA than whites. OA is more frequent in native Americans than in whites. Interphalangeal joint OA and especially hip OA are much less common in South African blacks than in whites in the same population.
- Age is the most powerful risk factor for OA. In a radiographic survey of women 45 years, only 2% had OA, between the ages of 45 and 64 years, however, the prevalence was 30% and for those 65 years it was 68%.
- The prevalence of clinician diagnosed arthritis is estimated at 46 million in the US and is projected to increase to nearly 67 million by 2030 of which 25 million are expected to report arthritis related activity limitations.

Incidence

The overall incidence of hip or knee OA is approximately 200 per 100,000 person years. Approximately one half million symptomatic new cases of OA are estimated to occur annually in the US.

Risk factors for OA

- ✓ Age
- ✓ Repetitive stress eg. Vocational
- ✓ Female sex
- ✓ Obesity
- ✓ Race Congenital/ developmental defects
- ✓ Genetic factors prior inflammatory joint disease
- ✓ Major joint trauma
- ✓ Metabolic/endocrine disorders

Developmental stages of Osteoarthritis

A. Destruction of the cartilage

- Proteolytic damage to the cartilage matrix

B. Inflammation of the synovial membrane

- Fibrillation and erosion of the cartilage surface and release of degradation products from the synovial fluid.

C. Remodeling of subchondral bone

- The synovial cells consume the degradation products. Production of inflammatory proteases and cytokines.

Fig No.5.1: A Osteoarthritic joint

Fig No.5.2: Normal and OA knee joint

Fig No.5.3:A cartilage eroded hip

Physiopathology of Osteoarthritis

The physiopathology of OA is not completely understood, but progress is being made.

- Under normal conditions, the components of the cartilage matrix are gradually replaced. Chondrocytes are the cells responsible for this metabolism in which synthesis(anabolism) and destruction (catabolism) are balanced in a coordinated way.
- When this process is altered, a series of changes occurs in the morphological and biomechanical characteristics of cartilage that make it fail to perform its function.
- Protease inhibitors and anti-inflammatory cytokines participate in the anabolic process, where the final aims are the formation of the extracellular matrix and cell proliferation.
- Pro-inflammatory cytokines and proteases participate in the catabolic process, which results in the destruction of the cartilage matrix and a reduction of cell proliferation.

Signs and symptoms

Nearly all patients have pain in the affected joints, with the hands, knees, and hips being the most common locations with motion, but pain in late disease can occur with rest.

- Joint stiffness resolves with motion; recurs with rest
- Joint stiffness with or without joint enlargement
- Crepitus, a crackling or grating sound heard with joint movement that is caused by irregularity of joint surfaces may be present.
- Limited range of motion that may be accompanied by joint instability.
- Late stage disease is associated with joint deformity

Diagnosis

In general, the disease can be detected because of its clinical and radiological signs. Some of the methods used to detect the clinical manifestations of Osteoarthritis include:

- Measuring pain using Huskisson's visual analogue scale, WOMAC (Western Ontario and Mc Master Universities) **Osteoarthritis Index**
- Measuring functional status
- Joint fluid aspiraton
- Radiology
- Other tests include: Nuclear Magnetic Resonance(NMR), Ultrasound, Bone gammagram, CT scan, Arthroscopy
- A system of Radiographic Grading of osteoarthritis is also used. It was developed by Kellgren and Lawrence and key in current radiological assessment of osteoarthritis.

Grades of Osteoarthritis

Table No.: 5.1 Grades of OA

Grade	Classification	Description
0	Normal	No characteristic symptoms of osteoarthritis
1	Doubtful	Indications of osteophytes Significance doubtful
2	Minimal	Definite osteophytes
3	Moderate	Moderate narrowing of joint space
4	Severe	Joint space very narrow, with subchondral bone sclerosis

Treatment

The most common symptom associated with OA is pain, which leads to decreased function and motion. Pain relief is the primary objective of medication therapy.

Non Pharmacologic measures

Non pharmacologic therapy is the foundation of the pharmaceutical care plan and should be initiated before or concurrently with pharmacologic therapy.

- Reduction of joint loading

- Patellar taping
- Thermal modalities
- Exercise
- Wedged insoles/orthoses
- Diet

Pharmacologic Therapy

Drug therapy for OA today is palliative, no pharmacologic agent has been shown to prevent, delay the progression of, or reverse the pathologic changes of OA in humans. It includes the following,

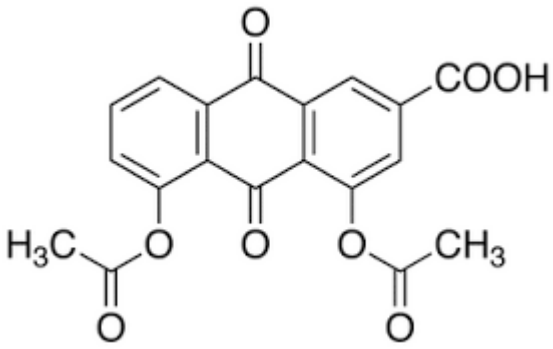
- NSAID and Acetaminophen
- Glucocorticoid injection
- Intraarticular injection of hyaluronan
- Opiods
- Rubefacients/Capsaicin
- Orthopedic surgery
- Glucosamine, chondroitin sulfate
- Cartilage regeneration

DIACEREIN[67-71]

Diacerein is a newly introduced one of the symptomatic slow acting drug for osteoarthritis. It is a semisynthetic anthraquinone derivative from certain plants. It belongs bone metabolism pharmacological group.

PHYSICOCHEMICAL PROPERTIES

Structure



Synonym : Diacetyl rhein

Description: A yellow fine powder.

Molecular formula :$C_{19}H_{12}O_8$

Molecular weight: 368.3

Chemical name: 9,10-dihydro-4,5-dihydroxy- 9,10- dioxo-2-anthranoic acid diacetate.

Category: Anti Rheumatic agent

Description : A yellow coloured powder

Solubility: Soluble in DMSO

Storage: Store at room temperature(25-30^{o}c)

PHARMACOKINECTICS:

Absorption:

Oral bioavailability of DCN is 35% to 56%. Concurrent intake of food delays the live to peak concentration from 2.4 hours to 5.2 hours but it is associated with a 25% in absorption. Therefore DCN is given with food.

Distribution:

Total protein binding is 99% to plasma albumin and in a lesser percentage to lipoprotein and gamma immunoglobulin. It achieves synovial fluid concentration of 0.3 to 3 mg/liter**.**

Metabolism:

DCN is metabolised extensively (100%) in liver following oral dosing to the deacetylation active metabolite rhein, prior to entering systemic circulation . Major active metabolite include rhein glucoronide and rhein sulphate with half life being 7 to 8 hours.

Excretion:

Urinary excretion of DCN in the form of its metabolities has ranged from 35% to 60% with approximately 20% as free rhein and 80% as conjugates of rhein.

PHARMACODYNAMICS:

Anti-osteoarthritic and a cartilage stimulating properties have been done *in-vitro* and in animal studies. DCN and its metabolities have been shown to inhibit the production of interleukin-1 beta by human monocytes and the effects of the cytokines on chondrocytes *in-vitro*. They exerts chondroprotective effect in cultured articular cartilage and reduce severity of cartilage, bone synovial membrane damage in osteoarthritis. Therefore they appear to be some inhibitory effects in leucocyte migration and activation, contributing to the weak anti inflammatory activity of drug, studies indicate that DCN does not block the synthesis of prostaglandin, the thromboxane and leukotrienes but may actually stimulate prostaglandin synthesis, especially PGF-2, a prostaglandin with cytoprotective effect in the gastric mucosa.

MECHANISM OF ACTION:

DCN in therapeutic doses inhibit the stimulation of interleukin-1 beta production and production of nitrous oxide. It also significantly reduces secretory of pathological changes of osteoarthritis compared to placebo and increases the expression of transforming growth factor (TGF)-beta 1 and (TGA)-beta 2, with potential cartilage repairing properties. DCN does not alter renal or platelet COX activity and may therefore be tolerated by patients with prostaglandin dependent renal function.

INDICATION:

DCN is indicated for the treatment of osteoarthritis of hip and knee.

DOSAGE AND ADMINISTRATION:

DCN should not be taken below 15 years of old as no clinical studies have been undertaken in the age group. The usual dosage of DCN is 50mg twice daily , after meals.

SIDE EFFECTS:

- Diarrhoea
- Epigastric pain
- Rashes
- Pruritus
- Nausea
- Vomiting
- Discolouration of urine

CONTRAINDICATIONS: Pregnancy, Lactation, Hypersensitivity to anthraquinone derivatives.

INTERACTIONS:

- Absence of interaction with drugs such as
- Warfarin
- Tolbutamide

- Aspirin
- Chlorpromazine
- Indomethacin

ADVERSE REACTIONS

The common reported adverse reaction was acceleration of the time of the intestinal transit (diarrhoea 37% of patients) few cases of abdominal pain have been describes, the medication of the dose in the initial periods of the treatment (2 to 4 hrs) has allowed to surpass or to diminish these adverse events.

Other adverse effects are urine discolouration in 14.4% cases and single case of hypokalemia, hepatotoxicity resulting into acute hepatitis and fatal toxic epidermal necrolysis(Lyell's syndrome)

AVAILABLE MARKETED PRODUCTS

- Dyserin
- Hilin
- Rasin
- Arcerin
- Artifit
- Ostogard

EUDRAGIT RS 100[72]

1.Nonproprietary names

BP:Ammonio methacrylate copolymer(Type B); USP-NF: Ammonio methacrylate

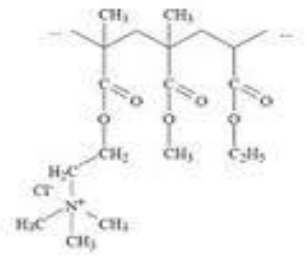
2.Synonyms

Eudragit, eastacryl,kollicoat MAE,Polymeric methacrylates, Acryl-EZE

3.Chemical name

Poly(ethyl acrylate,methyl methacrylate, trimethylammonioethyl methacrylate chloride).

4.Structural formula



5.Molecular weight is >100000

6.Description

Eudragit RS100 are ammonio methacrylate copolymers synthesized from acrylic acid and methacrylic acid esters. It occurs as fine granules with a slight amine like odour.

7.Solubility

Eudragit RS100 is soluble in acetone, alcohols, dichloromethane,ethylacetate and are water insoluble.

8.Functional category

Film forming agent, tablet binder, tablet diluents

9.Applications in Pharmaceutical formulation

Eudragit RS100 is used to form water insoluble film coats for sustained release products. It is also used as binders in both aqueous and organic wet granulation processes. They may also be used in direct compression processes and form the matrix layers of transdermal delivery systems. It is used to control the release of an active substance from a tablet matrix.

ETHYL CELLULOSE[72]

1 .**Nonproprietary Names**

BP: Ethylcellulose PhEur: Ethylcellulosum USPNF: Ethylcellulose

2 .**Synonyms**

Aquacoat ECD; Aqualon; E462; Ethocel; Surelease.

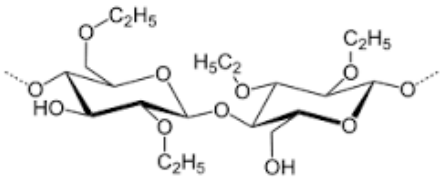
3 .**Chemical Name and CAS Registry Number**

Cellulose ethyl ether [9004-57-3]

4 **.Empirical Formula and Molecular Weight**

Ethylcellulose with complete ethoxyl substitution (DS = 3) is $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$ where n can vary to provide a wide variety of molecular weights. Ethylcellulose, an ethyl ether of cellulose, is a long-chain polymer of b-anhydroglucose units joined together by acetal linkages.

5 .**Structural Formula**



6 .**Functional Category**

Coating agent; tablet binder; tablet filler; viscosity-increasing agent.

7 .**Description**

Ethylcellulose is a tasteless, free-flowing, white to light tan colored powder.

8. **Solubility**:

Ethylcellulose is practically in soluble in glycerine ,propyleneglycol, and water. Ethylcellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%).

9 .**Applications in Pharmaceutical Formulation or Technology**

Ethylcellulose is widely used in oral and topical pharmaceutical formulations. The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules.Ethylcellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation.

POLYVINYL ALCOHOL[72]

1 .**Nonproprietary Names**

PhEur: Poly(vinylis acetas) USP: Polyvinyl alcohol

2 .**Synonyms**

Airvol; Alcotex; Elvanol; Gelvatol; Gohsenol; Lemol; Mowiol; Polyvinol; PVA; vinyl alcohol polymer.

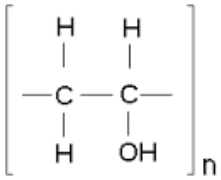
3 .**Chemical Name and CAS Registry Number**

Ethenol, homopolymer [9002-89-5]

4 .**Empirical Formula and Molecular Weight**

$(C_2H_4O)_n$ 20000–200000 Polyvinyl alcohol is a water-soluble synthetic polymer represented by the formula $(C_2H_4O)n$. The value of n for commercially available materials lies between 500 and 5000, equivalent to a molecular weight range of approximately 20000–200000.

5 .**Structural Formula**



6 **.Functional Category**

Coating agent; lubricant; stabilizing agent; viscosity-increasing agent.

7 **.Description**

Odourless, white to cream coloured granular powder

8.**Solubility**

Soluble in water, slightly soluble in ethanol(95%), insoluble in organic solvents

9 .**Applications in Pharmaceutical Formulation or Technology**

Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulations. It is used as a stabilizing agent for emulsions (0.25–3.0% w/v). Polyvinyl alcohol is also used as a viscosity-increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solutions for lubrication purposes, in sustained-release formulations for oral administration, and in transdermal patches. Polyvinyl alcohol may be made into microspheres when mixed with a glutaraldehyde solution.

TRIETHYL CITRATE[72]

1 .**Nonproprietary Names**

BP: Triethyl citrate PhEur: Triethylis citras USPNF: Triethyl citrate

2 .**Synonyms**

Citric acid, ethyl ester; Citroflex 2; Citrofol AI; E1505; Hydagen CAT; TEC.

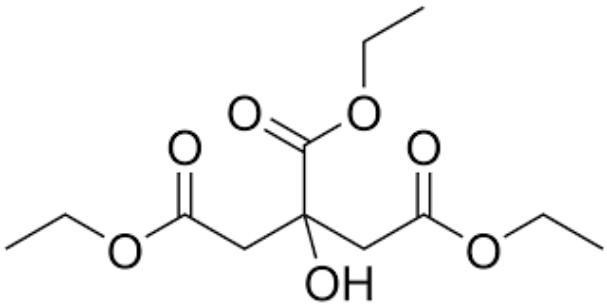
3 .**Chemical Name and CAS Registry Number**

2-Hydroxy-1,2,3-propanetricarboxylic acid, triethyl ester [7793-0]

4 .**Empirical Formula and Molecular Weight**

$C_{12}H_{20}O_7$ and 276.29

5 .**Structural Formula**



6 .**Functional Category**

Plasticizer.

7 .**Applications in Pharmaceutical Formulation or Technology**

Triethyl citrate and the related esters acetyl triethyl citrate, tributyl citrate, and acetyltributyl are used to plasticize polymers in formulated pharmaceutical coatings. The coating applications include capsules, tablets, beads, and granules for taste masking, immediate release, sustained release, and enteric formulations. Triethyl citrate is also used as a direct food additive for flavouring, for solvency, and as a surface active agent.

8. **Description**

Triethyl citrate is a clear, odourless, practically colourless, oily liquid.

MAGNESIUM STEARATE[72]

1 **Nonproprietary Names**

BP: Magnesium stearate JP: Magnesium stearate PhEur: Magnesii stearas USPNF: Magnesium stearate

2 **Synonyms**

Magnesium octa decanoate; Octadecanoic acid, Magnesium salt; Stearic acid, Magnesium salt.

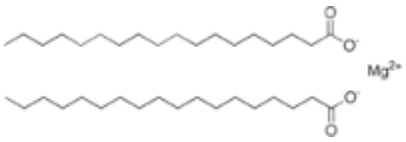
3 **Chemical Name and CAS Registry Number**

Octadecanoic acid magnesium salt [557-04-0]

4 **Empirical Formula and Molecular Weight**

$C_{36}H_{70}MgO_4$ and 591.34

5 **Structural Formula**



6 **Functional Category**

Tablet and capsule lubricant.

7 **Applications in Pharmaceutical Formulation or Technology**

Magnesium stearate is widely used in cosmetics, foods, and pharmaceutical formulations. It is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25% and 5.0% w/w. It is also used in barrier creams.

8.**Solubility**

Practically insoluble in ethanol, ethanol(95%),ether and water; slightly soluble in warm benzene and warm ethanol (95%).

9. **Description**

Magnesium stearate is a very fine, light white, precipitated or milled, impalpable powder of low bulk density, having a faint odour of stearic acid and a characteristic taste. The powder is greasy to the touch and readily adheres to the skin.

MATERIALS

Table No. 8.1 The list of drug and excipient, their manufacturer and role in the present study

INGREDIENTS	MANUFACTURER/SUPPLIER	ROLE IN FORMULATION
Diacerein	Madras Pharmaceuticals, Chennai	Active ingredient
Eudragit RS 100	Evonik India Pvt. Ltd, Mumbai	Polymer
Ethylcellulose	Caplin Point, Puducherry	Polymer
Polyvinyl alcohol	S D fine chem.. Ltd, Mumbai	Stabilizing agent
Triethyl citrate	Himedia Laboratories Pvt. Ltd.	Plasticizer
Dichloromethane	Chenchems	Solvent
Magnesium stearate	Kniss laboratories, Chennai	Lubricant
Potassium dihydrogen phosphate	Qualigens	Reagent
Sodium hydroxide	Chenchems	Reagent

EQUIPMENTS USED FOR PREPARATION OF MICROSPONGES

Table No.8.2 Equipments used in the formulation and evaluation of microsponges.

NAME OF THE EQUIPMENT	MANUFACTURER/ SUPPLIER
Electronic balance	Asha Scientific Company, Mumbai.
High speed homogenisor	Remi electrotechnik, Vasai
Hot air oven	Mc Dalal, Chennai
Optical Microscope	Sigma Scientific Instrumentation, Chennai
pH meter	Mc Dalal, Chennai
Dissolution apparatus	Campbell electronics, Mumbai
UV spectrophotometer	Shimadzu, Japan
FT-IR spectrophotometer	Shimadzu, Japan
SEM Analyser	Hitachi, Japan

METHODOLOGY

DRUG-EXCIPIENT COMPATIBILITY STUDY

The drug and excipients selected for the formulation are evaluated for physical and chemical compatibility studies.

PHYSICAL COMPATIBILITY STUDY [30]

100mg each of powder drug, Polymer, PVA were weighed. Individual drug, polymer, PVA along with admixture of drug and excipients in airtight screw cap amber colored vials., kept at room temperature as well as at $40^0C \pm 2^oC$ / 75% ± 5 % RH for 30 days.

CHEMICAL COMPATIBILITY STUDY [23]

Infrared spectroscopy was conducted using FT-IR spectrophotometer and the spectrum was recorded in the wave number region of 4000 to 400 cm^{-1}. The procedure consisted of dispersing the sample (drug alone, mixture of drug and excipients and the optimized formulation) in Potassium bromide and compressed into discs by applying a pressure of 5 tons for 5 minutes in a hydraulic press. The pellet was placed in the light path and the spectrum was recorded.

STANDARD CURVE FOR DIACEREIN [45, 62]

10 mg of diacerein was weighed and transferred to 100 ml of volumetric flask. The drug was dissolved in 2 ml DMSO and the volume was made up to 100 ml using phosphate buffer pH 6.8 to obtain a stock solution of 100 µg/ml (stock solution I). One ml of this stock solution was again diluted with phosphate buffer pH 6.8 up to 10 ml to obtain a solution of 10 µg/ml (stock solution II). From stock solution II of 2, 4, 6, 8, 10 ml were transferred to a series of 10 ml volumetric flasks. The volume was made up with phosphate buffer pH 6.8. The absorbances of these solutions was measured at 258 nm against blank.

FORMULATION DEVELOPMENT

Formulation of Diacerein Microsponges

Diacerein microsponges were prepared by using Quasi emulsion solvent diffusion technique with Polymers like Eudragit RS 100 and Ethyl cellulose at different drug to polymer ratios.

Procedure to formulate microsponges [40]

1. Internal phase: Polymer (Eudragit RS 100/ Ethyl Cellulose) was dissolved in 5ml of dichloromethane. Diacerein was added and mixed well until it gets dissolved completely and to which triethyl citrate was added to facilitate plasticity.
2. External phase: Accurately weighed PVA is added to distilled water to form clear solution.
3. The internal phase was added to external phase and stirred for 2 hours at room temperature.
4. The mixture was filtered to separate microsponges and were dried in an air heated oven at 40^{o}C for 12 hr and stored for subsequent investigation.

Fig. No.: 8.1 Formulation of microsponges

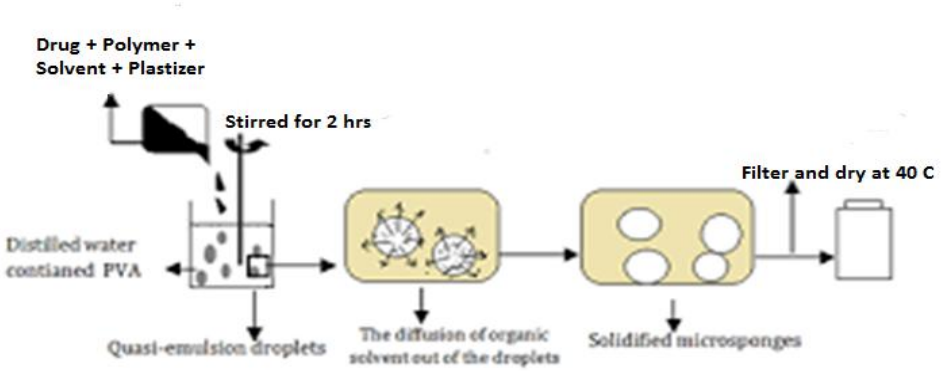


Table No.:8.3 Composition of Diacerein microsponges

Ingredients	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Diacerein (mg)	200	400	600	800	1000	200	400	600	800	1000
Eudragit RS 100 (mg)	200	200	200	200	200	-	-	-	-	-
Ethyl cellulose (mg)	-	-	-	-	-	200	200	200	200	200
Polyvinyl alcohol (mg)	200	200	200	200	200	200	200	200	200	200
Triethyl citrate (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dichloromethane (ml)	5	5	5	5	5	5	5	5	5	5
Distilled water (ml)	100	100	100	100	100	100	100	100	100	100

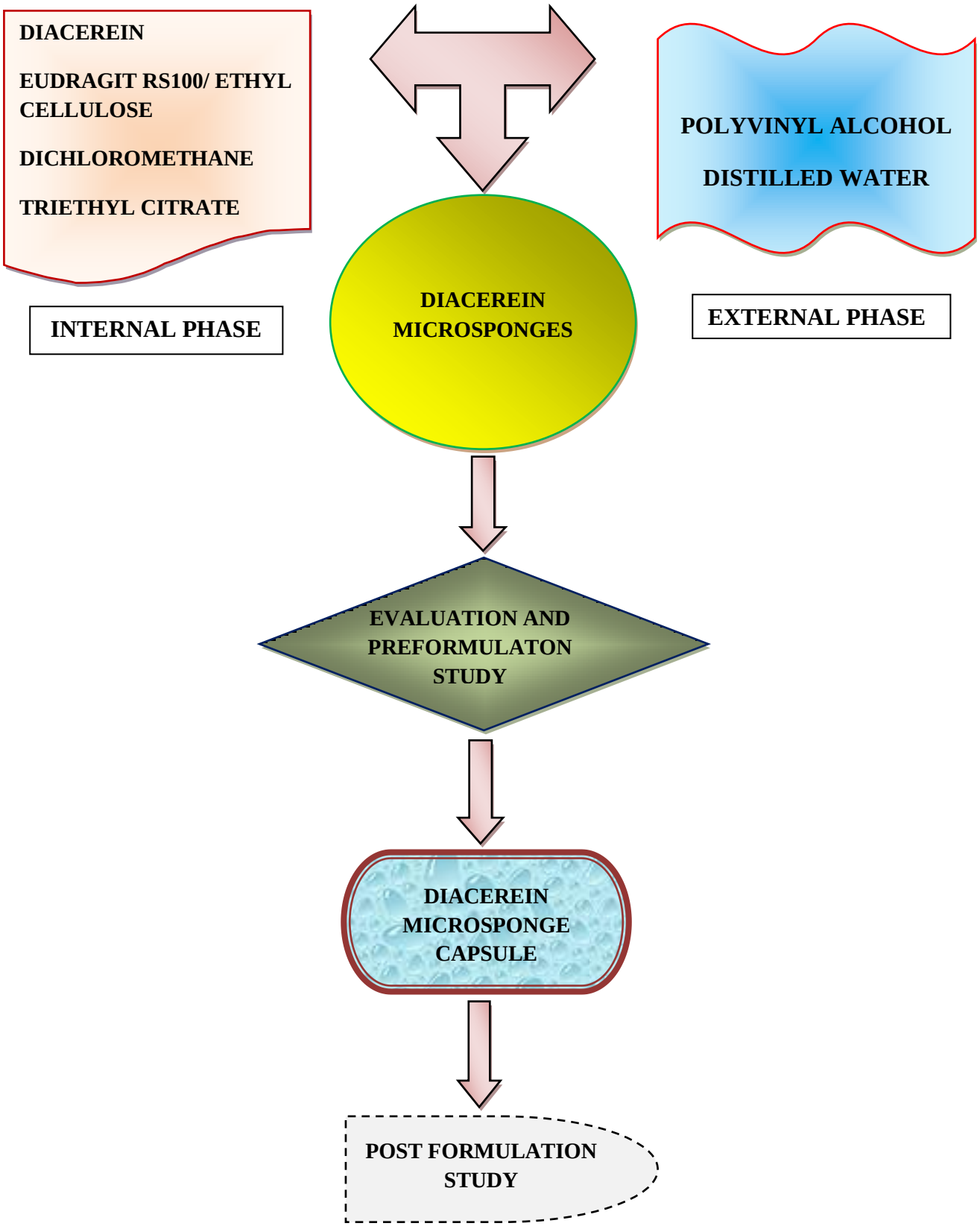


Fig.No.8.2: SCHEMATIC PRESENTATION OF DIACEREIN MICROSPONGE CAPSULES

EVALUATION OF MICROSPONGES

PRODUCTION YIELD[34]

Percentage yield can be determined by calculating the initial weight of raw materials and the finally obtained weight of microsponges. Percentage yield can be calculated by using the formula:

$$\text{Production yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

LOADING EFFICIENCY [27]

The microsponges was determined spectrophotometrically (λmax = 258 nm). A sample of DCN microsponges (100 mg) was dissolved dissolved in 100 ml of phosphate buffer (pH 6.8) and kept for overnight. The drug content was determined and expressed as actual drug content in microsponge. The loading efficiency (%) of the microsponges was calculated according to the following equation,

$$\text{Loading efficiency} = \frac{\text{Actual drug content in microsponges}}{\text{Theoretical drug content}} \times 100$$

PARTICLE SIZE ANALYSIS [39]

Determination of the average particle size of Diacerein loaded microsponges was determined with an optical microscope using a calibrated ocular and stage micrometer. A minute quantity of microsponges was spread on a clean glass slide with a drop of liquid paraffin and a cover slip is placed on it. The average particle size was calculated by measuring 100 particles of each batch.

$$dav = \sum nd / \sum n$$

Where, dav is the average diameter of particles (μm), n is number of particles per group, and d is the middle value (μm).

SURFACE MORPHOLOGY [41]

Scanning Electron Microscopy of optimized microsponge formulation was carried to determine the surface morphology. The sample was mounted directly onto the SEM sample holder using double sided sticking tape and images were recorded at different magnifications at acceleration voltage of 10 kV using scanning electron microscope.

***In-vitro* DRUG RELEASE STUDY**[24]

In-vitro release rate studies of microsponges were carried out by filling equivalent amount of microsponge in capsules placed in the basket containing phosphate buffer pH 6.8 was used as medium and rotated at 50 rpm. Samples was withdrawn and determined by spectrophotometrically at 258 nm.

PREFORMULATION FOR CAPSULES

FLOW PROPERTY MEASUREMENTS

The flow properties of powders are critical for an efficient tabletting and capsule filling operation. A good flow of the powder or granules is necessary to assure efficient mixing and acceptable weight uniformity for the compressed tablet and capsules. The flow property measurements include bulk density, tapped density, angle of repose, compressibility index and Hausner's ratio. The flow property measurements of microsponges are determined .

a) **BULK DENSITY** (ρ_b)[73]

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and initial weight was noted. This initial volume was called the bulk volume. From this the bulk density was calculated according to the formula mentioned below. It is expressed in g/ml and is given by

$$\rho_b = M / V_b$$

Where, M and V_b are mass of powder and bulk volume of the powder respectively.

b) **TAPPED DENSITY**(ρ_t)[73]

It is the ratio of weight of the powder to the tapped volume of powder. The powder was introduced into a measuring cylinder with the aid of funnel and tapped for 500 times on a wooden surface at a 2 sec interval and the volume attained is the tapped volume.

$$\rho_t = M / V_t$$

Where, M and V_t are mass and tapped volume of the powder respectively. It is expressed in g/ml.

c) **ANGLE OF REPOSE** (θ)[73]

The flow properties were characterized in terms of angle of repose, Carr's index and Hausner's ratio. For determination of angle of repose (θ), the drug and the blend were poured through the walls of a funnel, which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above hard surface. The drug or the blends were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. Angle of repose was calculated using following equation.

$$\theta = \tan^{-1}(h/r)$$

Where, h = height of pile in cm; r = radius of pile in cm.

d) CARR'S INDEX (OR) % COMPRESSIBILITY[73]

It indicates powder flow properties. It is measured for determining the relative importance of interparticulate interactions. It is expressed in percentage and is given by

$$CI = \frac{\rho_t - \rho_b}{\rho_t} \times 100$$

Where, ρ_t and ρ_b are tapped density and bulk density respectively.

e) HAUSNER'S RATIO[73]

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

$$HR = \rho_t / \rho_b$$

Where, ρ_t and ρ_b are tapped density and bulk density respectively.

f) POROSITY (%)[74]

Porosity is defined as the ratio of the void volume to the bulk volume of a powder packing. It is calculated using true density value obtained from liquid displacement method. It gives total porosity since the void space determined takes into account the intraparticle space i.e pores and cracks within the particles. It is also given as

$$\varepsilon = 1 - \frac{\text{Bulk density}}{\text{True density}} \times 100$$

Table 8.4: Angle of Repose, Compressibility Index and Hausner's Ratio

Flow property	Angle of Repose(θ)	Compressibility Index(%)	Hausner's Ratio
Excellent	25-30	<10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26- 1.34
Poor	46-55	26-31	1.35-1.45
Very Poor	56-65	32-37	1.46-1.59
Very very Poor	>65	>38	>1.60

PREPARATION OF CAPSULE

The optimised microsponges were filled into "1" size capsule each containing 100mg equivalent of DCN.

Table No.:8.5 Preparation of diacerein microsponge capsules

Optimized formulation	Diacerein microsponges (mg) (equivalent to 100mg)	Magnesium stearate (mg) (3%)
F5	150	4.5

EVALUATION OF CAPSULE

UNIFORMITY OF WEIGHT [75]

Intact capsule were weighed. The capsule were opened without losing any part of the shell and contents were removed as completely as possible. The shell was washed with ether or other suitable solvent and the shell allowed to stand until the odour of the solvent was no longer detectable. The empty shell was weighed the procedure was repeated with a further 19 capsules. The average weight was determined. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation shown in table 8.6 and none deviates by more than twice that percentage.

Table No. 8.6: Uniformity of weight

Average weight of capsule contents	Percentage deviation
Less than 300mg	10
300mg or more	7.5

DISINTEGRATION TEST [75]

This test determines whether capsules disintegrate within a prescribed time when placed in a liquid medium under the prescribed experimental conditions.

A capsule was placed in each of the six tubes of the apparatus and one disc was added to each tube. The time in minutes taken for complete disintegration of the capsule with no palpable mass remaining in the apparatus was measured.

DRUG CONTENT[75]

Five capsules were selected randomly and the average weight was calculated. The powder is removed completely and equivalent amount of powder is made up to 100ml with phosphate buffer pH 6.8. 10ml of solution is diluted to 100ml using phosphate buffer pH 6.8 in separate standard flask. The absorbance of solution was recorded at 258nm.

In-vitro **DRUG RELEASE** [24]

In-vitro release studies of microsponges were carried out by filling equivalent amount of microsponge along with lubricant in capsules and placed in the basket containing phosphate buffer pH 6.8 was used as medium and rotated at 50 rpm. Samples was withdrawn and determined by spectrophotometrically at 258 nm.

Evaluation of optimized formulation

Effect of stirring rate on microsponges:[20]

The effect of stirring rate on the formulated microsponges were examined at different stirring rates of 800,1000,1200 rpm . The results of stirring rates on the mean particle size, production yield, drug content and *in-vitro* drug release were studied .

Effect of amount of inner phase solvent on microsponges: [20]

The effect of inner phase solvent on the formulated microsponges were examined at different solvent amounts of 5,10,15 ml. The effect of inner phase solvent

on the mean particle size, production yield, drug content and *in-vitro* drug release was studied.

Effect of external phase composition on microsponges: [20]

The effect of external phase on the formulated microsponges were examined at different concentration of PVA 0.2mg,0.3mg,0.4mg. The effect of external phase PVA on the mean particle size, production yield, drug content and *in-vitro* drug release was studied.

***In- vitro* anti-inflammatory test**: [76]

1 ml of sample solution was withdrawn during *in-vitro* drug release study at every one hour interval, thereafter subjected to *in-vitro* anti-inflammatory analysis. For the purpose of control, equal volume of distilled water was used. To each reaction mixture, 1 ml of bovine albumin(1% in distilled water) was transferred and pH was adjusted to 6.3 by using small amount of 0.1 N HCl. Samples were incubated for 30 min at 37^0C in the dark followed by incubation at 57^0C for 5 min. Reaction tubes were then cooled under running tap water and turbidity of all the samples were recorded spectrophotometrically at 660 nm. Percentage inhibition of albumin denaturation was calculated by using

$$\text{Percentage inhibition} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

RELEASE KINETICS OF THE OPTIMIZED FORMULATIONS[77]

To study the *in-vitro* release kinetics of the optimized formulation, data obtained from dissolution study were plotted in various kinetics models.

1. Zero order equation : The zero order release can be obtained by plotting cumulative % percentage drug released vs. time in hours

$$C=K_0t$$

Where, K_0= Zero order constant

t= Time in hours

2. First order equation:

The graph was plotted as log % cumulative drug remaining Vs time in hours.

$$\text{Log } C = \log C_0 - Kt/2.303$$

Where, C_0= Initial concentration of drug ,
K= First order,
t= Time in hours

3. Higuchi kinetics:

The graph was plotted with % cumulative drug released vs. square root of time.

$$Q = Kt\ ½$$

Where , K= constant reflecting design variable system (differential rate constant)
t= Time in hours .

The drug release rate is inversely proportional to the square root of time.

4. Hixon and Crowell erosion equation:

To evaluate the drug release with changes in the surface area and the diameter of particles, the data were plotted using the Hixon and Crowell rate equation. The graph was plotted by cube root of % drug remaining vs. time in hours.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC}\, t$$

Where , Qt= amount of drug released in time t,
Q_0= Initial Amount of drug ,
KHC= Rate constant for Hixon Crowell equation

5. Korsmeyer-Peppas equation: To evaluate the mechanism of drug release, it was further plotted in Korsmeyer-Peppas equation as log cumulative % of drug released Vs log time.

$$M_t/M_\alpha = Kt^n$$

Where , M_t/M_α = Fraction of drug released at time t

t = Release time

K= Kinetics constant (Incorporating structural and geometric characteristics of the formulation)

N= Diffusional exponent indicative of the mechanism of drug release.

Table No. 8.7: Diffusion exponent and solute release mechanism for cylindrical shape Diffusion

Diffusion coefficient	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45<n<0.89	Anamolous (non-fickian diffusion)
0.89	Case II transport
n>0.89	Super Case II transport

PHYSICAL COMPATIBILITY STUDY

Table No.9.1: Physical compatibility study

S. No.	Description and Conditions								
	Drug & Excipient	Initial	Room temperature (in days)			40^{o} C ± 2^{o}C / 75 % ± 5% RH (in days)		
			10	20	30	10	20	30
1	DCN	Yellow coloured powder	NC	NC	NC	NC	NC	NC
2	EUD	White coloured granules	NC	NC	NC	NC	NC	NC
3	EC	White coloured powder	NC	NC	NC	NC	NC	NC
4	PVA	White coloured powder	NC	NC	NC	NC	NC	NC
5	MS	White coloured powder	NC	NC	NC	NC	NC	NC
6	DCN+EUD	Yellow coloured powder	NC	NC	NC	NC	NC	NC
7	DCN+EC	Yellow coloured powder	NC	NC	NC	NC	NC	NC
8	DCN+PVA	Yellow coloured powder	NC	NC	NC	NC	NC	NC
9	DCN+MS	Yellow coloured powder	NC	NC	NC	NC	NC	NC

The physical compatilibity study was performed. There was no change of colour. Therefore the drug and excipients are physically compatible with each other. The excipients which are compatible with the drug were selected for formulation.

FT-IR STUDY

Interaction between the drug and excipients used in the formulation was studied. The results are as follows

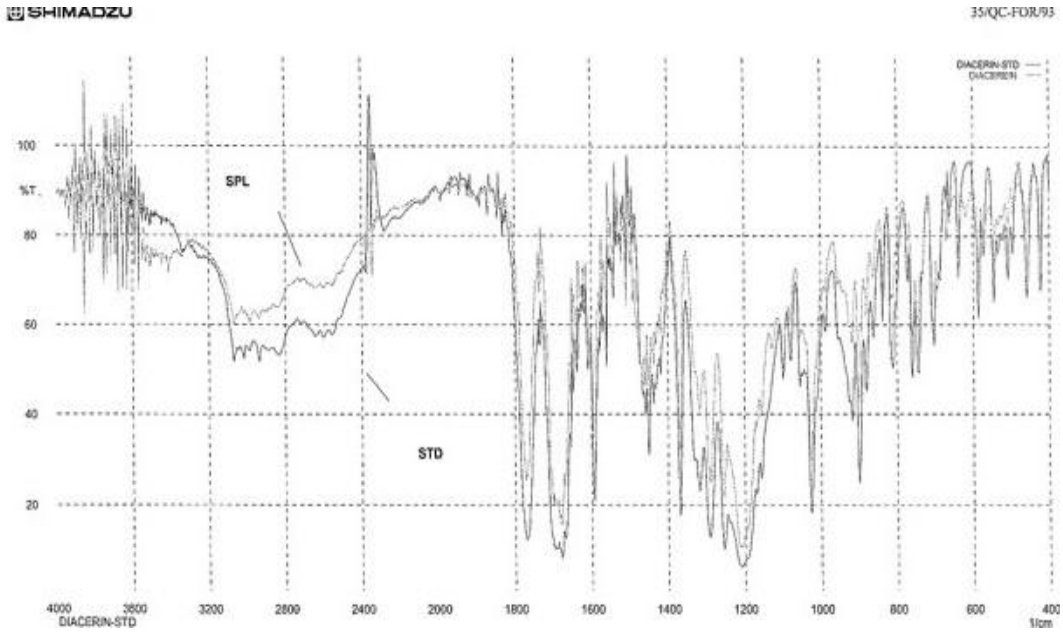


Fig No.9.1 : FT-IR Of Diacerein

Table No. 9.2: FT-IR Spectral interpretation of Diacerein

Wave number(cm^{-1})	**Types of vibration**
1766.67	C=O Stretching
2923.87	Ar-H Stretching
3440.76	O-H Stretching
1211.21	C-O Stretching

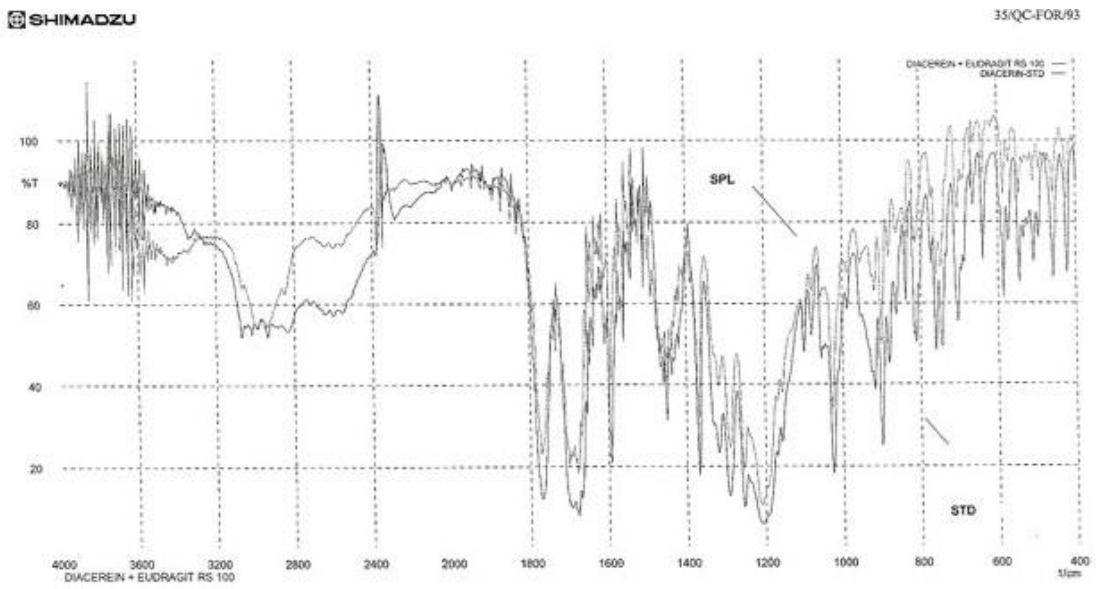


Fig No.9.2 : FT-IR Of Diacerein and Eudragit RS 100

Table No. 9.3: FT-IR Spectral interpretation of Diacerein and Eudragit RS 100

Wave number(cm^{-1})	Types of vibration
1766.67	C=O Stretching
2923.87	Ar-H Stretching
3440.76	O-H Stretching
1211.21	C-O Stretching

The peaks observed in the FT-IR spectrum showed no shift and no disappearance of characteristic peaks of drug. This suggests that there is no interaction between the drug and Eudragit RS 100.[79]

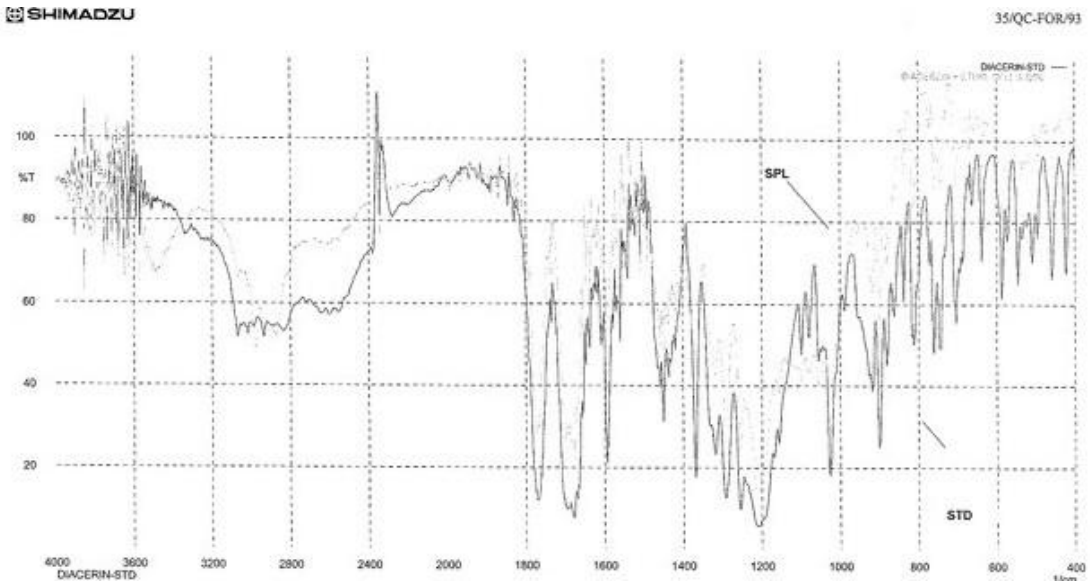


Fig No.9.3: FT-IR Of Diacerein and Ethyl Cellulose

Table No. 9.4: FT-IR Spectral interpretation of Diacerein and Ethyl Cellulose

Wave number(cm^{-1})	**Types of vibration**
1766.67	C=O Stretching
2923.87	Ar-H Stretching
3440.76	O-H Stretching
1211.21	C-O Stretching

The peaks observed in the FT-IR spectrum showed no shift and no disappearance of characteristic peaks of drug. This suggests that there is no interaction between the drug and Ethyl Cellulose. [79]

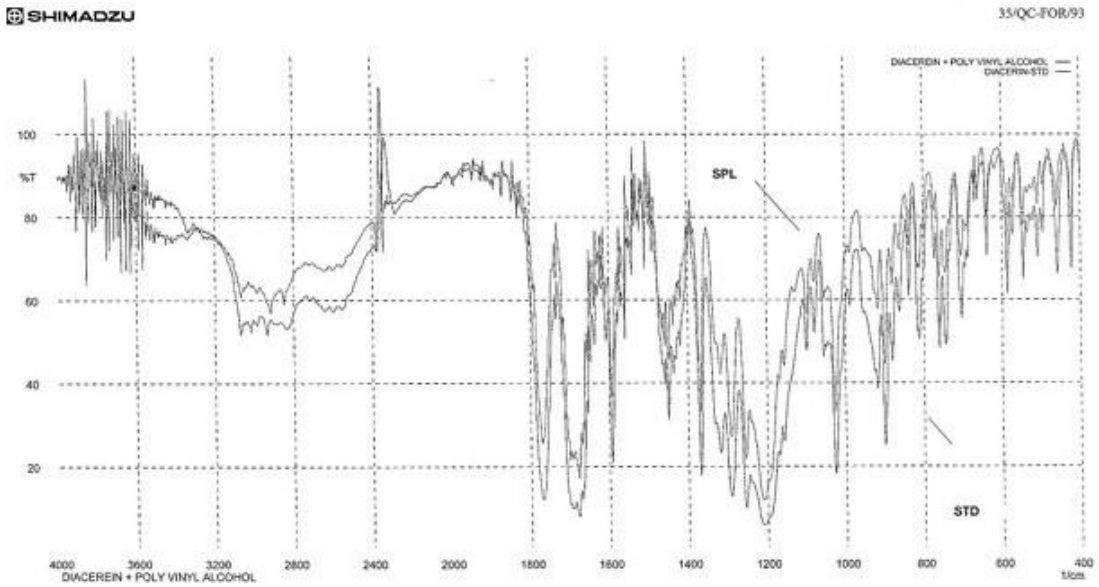


Fig No. 9.4: FT-IR Of Diacerein and Polyvinyl Alcohol

Table No. 9.5: FT-IR Spectral interpretation of Diacerein and Polyvinyl Alcohol

Wave number(cm^{-1})	Types of vibration
1766.67	C=O Stretching
2923.87	Ar-H Stretching
3440.76	O-H Stretching
1211.21	C-O Stretching

The peaks observed in the FT-IR spectrum showed no shift and no disappearance of characteristic peaks of drug. This suggests that there is no interaction between the drug and Polyvinyl Alcohol. [79]

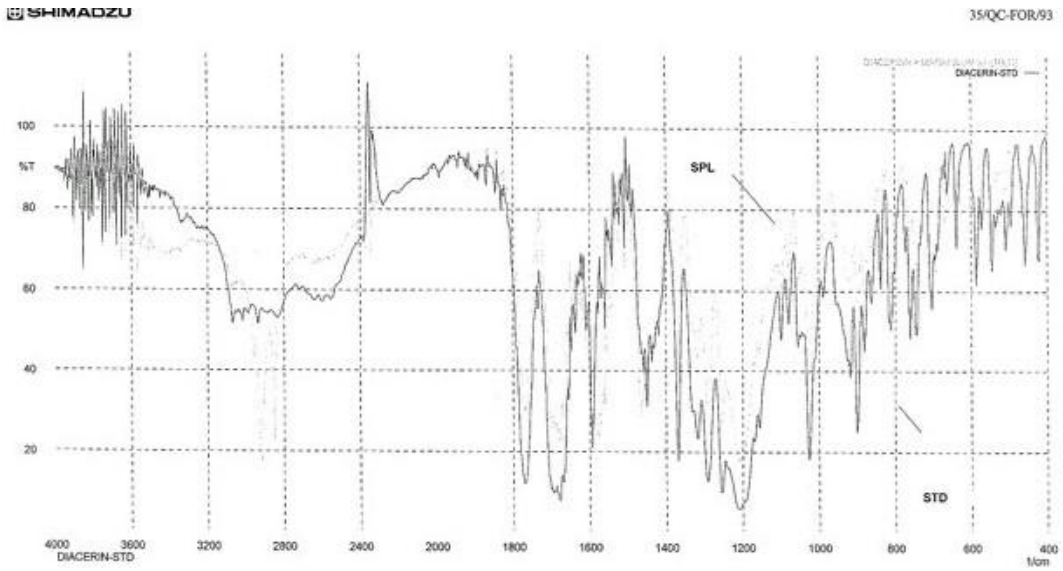


Fig No. 9.5: FT-IR Of Diacerein and Magnesium Stearate

Table No. 9.6: FT-IR Spectral interpretation of Diacerein and Magnesium Stearate.

Wave number(cm^{-1})	Types of vibration
1766.67	C=O Stretching
2923.87	Ar-H Stretching
3440.76	O-H Stretching
1211.21	C-O Stretching

The peaks observed in the FT-IR spectrum showed no shift and no disappearance of characteristic peaks of drug. This suggests that there is no interaction between the drug and Magnesium stearate. [79]

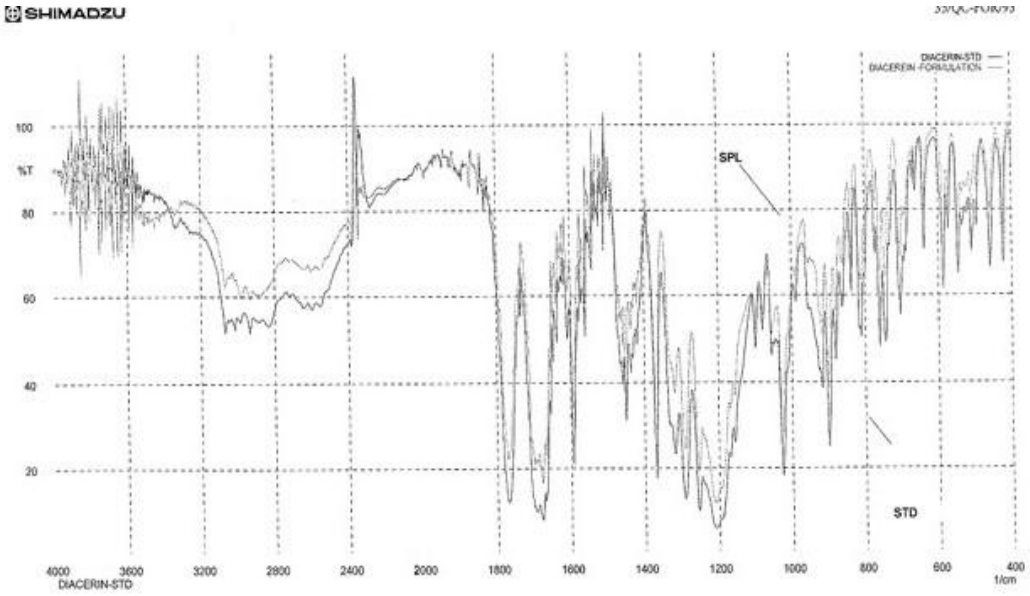


Fig No. 9.6: FT-IR Of Diacerein and powder blend

Table No. 9.7: FT-IR Spectral interpretation of Diacerein and Powder blend (Eudragit RS 100)

Wave number(cm^{-1})	Types of vibration
1766.67	C=O Stretching
2923.87	Ar-H Stretching
3440.76	O-H Stretching
1211.21	C-O Stretching

From the FT-IR spectra, it is clearly evident that the physical mixtrures of DCN with different excipients showed the presence of DCN characteristics bands at their same wave number. This indicates the absence of chemical interaction between the drug and the excipients. [79]

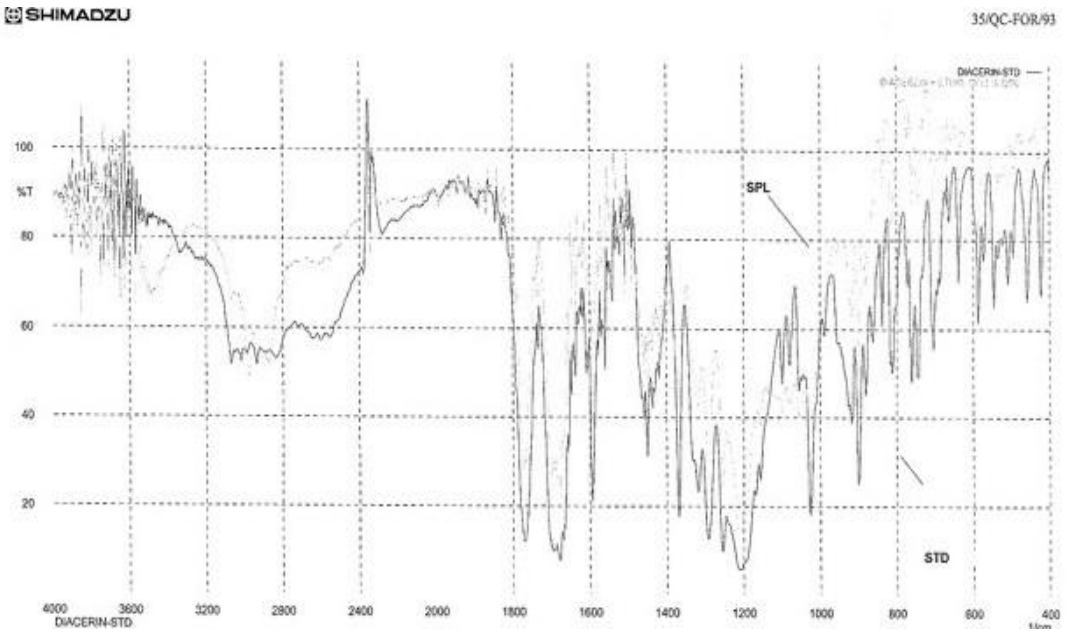


Fig No.9.7: FT-IR of Diacerein and powder blend

Table No. 9.8: FT-IR Spectral interpretation of Diacerein and Powder blend(Ethyl cellulose)

Wave number(cm^{-1})	**Types of vibration**
1766.67	C=O Stretching
2923.87	Ar-H Stretching
3440.76	O-H Stretching
1211.21	C-O Stretching

From the FT-IR spectra, it is clearly evident that the physical mixtrures of DCN with different excipients showed the presence of DCN characteristics bands at their same wave number. This indicates the absence of chemical interaction between the drug and the excipients. [79]

STANDARD GRAPH OF DIACEREIN[45]

Solutions of Diacerein in Phosphate buffer pH 6.8 were suitably diluted to give varying concentrations of 2-10µg/ml. The absorbances was measured at 258nm and the values are given in Table No.9.9

Table No.9.9: Absorbance of Diacerein in Phosphate buffer pH 6.8

S.No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.209±0.005
3	4	0.407±0.012
4	6	0.599±0.010
5	8	0.805±0.013
6	10	1.021±0.020

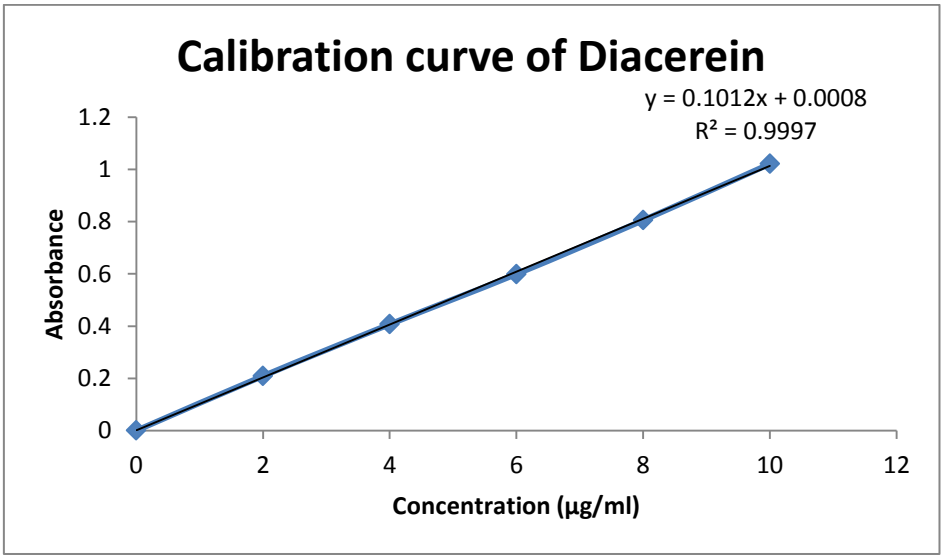


Fig. No. 9.8: Standard graph of diacerein in phosphate buffer pH 6.8

It was found that the solutions show linearity (R^2=0.999)in absorbance at a concentration of 2-10µg/ml and obeys Beer Lambert's law.

SCANNING ELECTRON MICROSCOPY

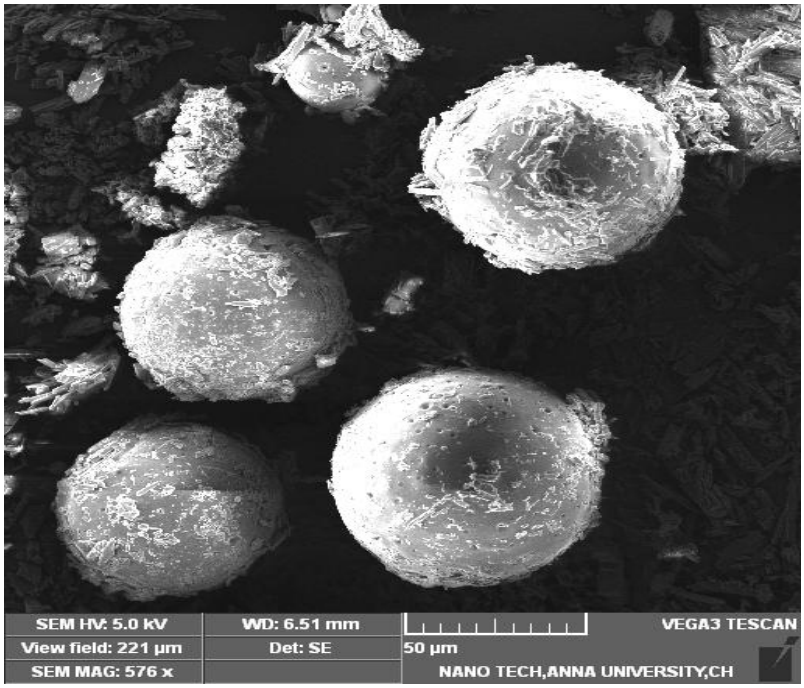


Fig. No. 9.9: Scanning Electron Microscopy of F5

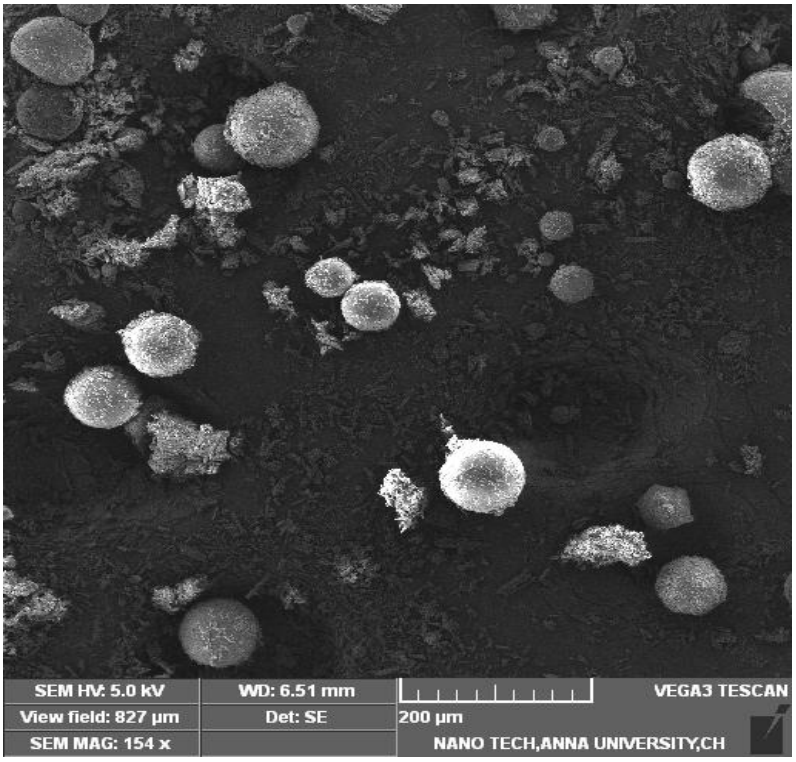


Fig. No. 9.10 : Scanning Electron Microscopy of F9

The shape and surface morphology of optimized microsponges(F5,F9) was observed in SEM. It shows that the microsponges were spherical, porous and uniform.[21]

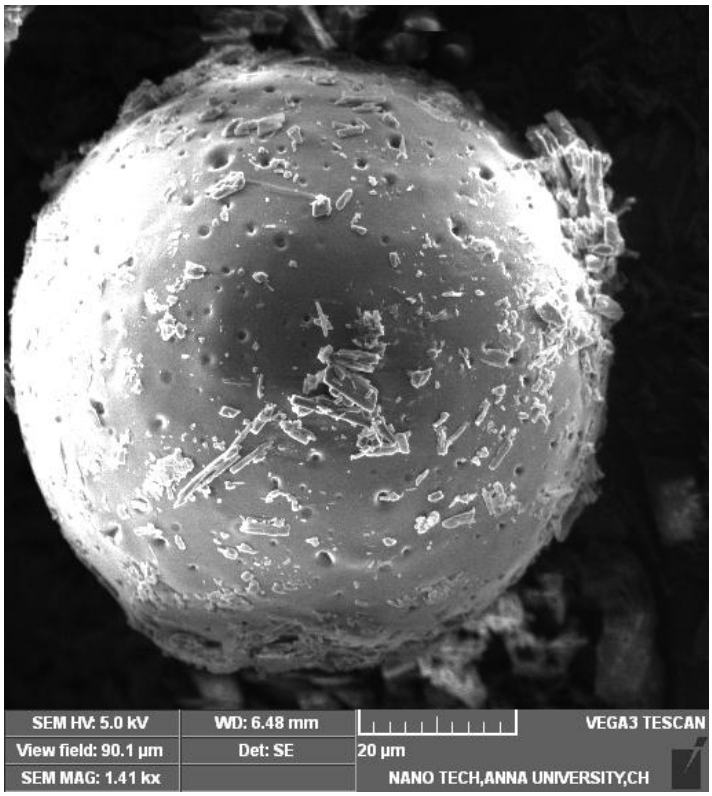


Fig. No.9.11: A porous Microsponge

EVALUATION OF MICROSPONGES

PERCENTAGE YIELD

Table No. 9.10: Percentage yield of Diacerein microsponges

Formulation code	Theoretical yield (g)	Practical yield (g)	Percentage yield (%)
F1	0.6	0.393	65.60
F2	0.8	0.536	67.09
F3	1	0.781	78.10
F4	1.2	1.000	83.34
F5	1.4	1.176	84.02
F6	0.6	0.330	56.38
F7	0.8	0.537	67.60
F8	1	0.739	73.91
F9	1.2	0.934	77.83
F10	1.4	1.199	85.64

Microsponges were prepared and their Percentage yield was calculated. They were found to be in the range of 56.38% to 85.64%. It shows increasing drug:polymer ratio increased the percentage yield. The results correspond to earlier reports.[39]

LOADING EFFICIENCY

Table No.9.11: Percentage loading efficiency

Formulation code	Loading efficiency (%)
F1	67.42
F2	75.81
F3	90.76
F4	92.53
F5	95.75
F6	75.80
F7	86.51
F8	90.01
F9	92.44
F10	95.44

% Loading efficiency ranged from 67.42 to 95.75% . Highest loading efficiency was found for the formulation F5 and F10. This shows that increasing drug:polymer ratio increased loading efficiency. The results correspond to earlier reports [39]

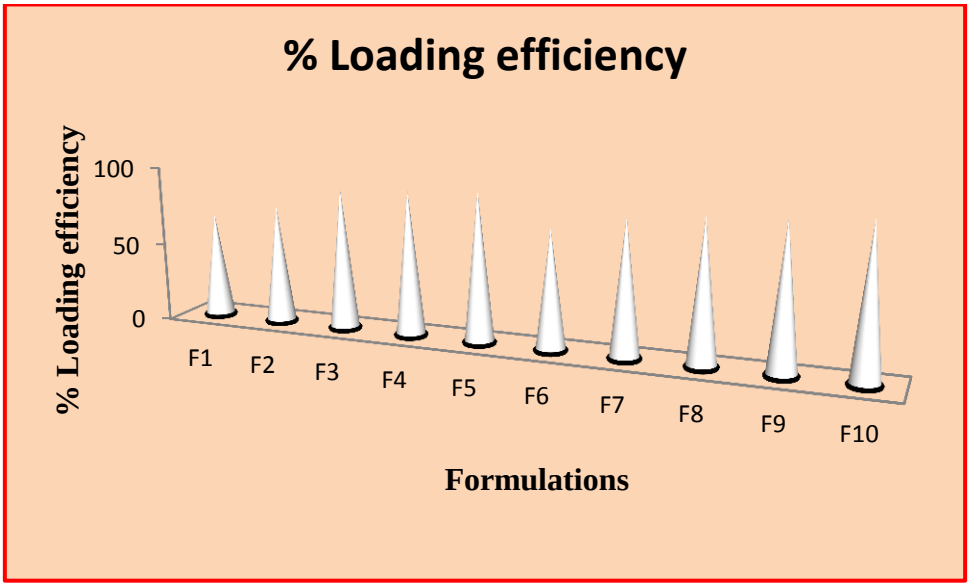


Fig. No. 9.12 : Percentage loading efficiency

PARTICLE SIZE DISTRIBUTION BY OPTICAL MICROSCOPY

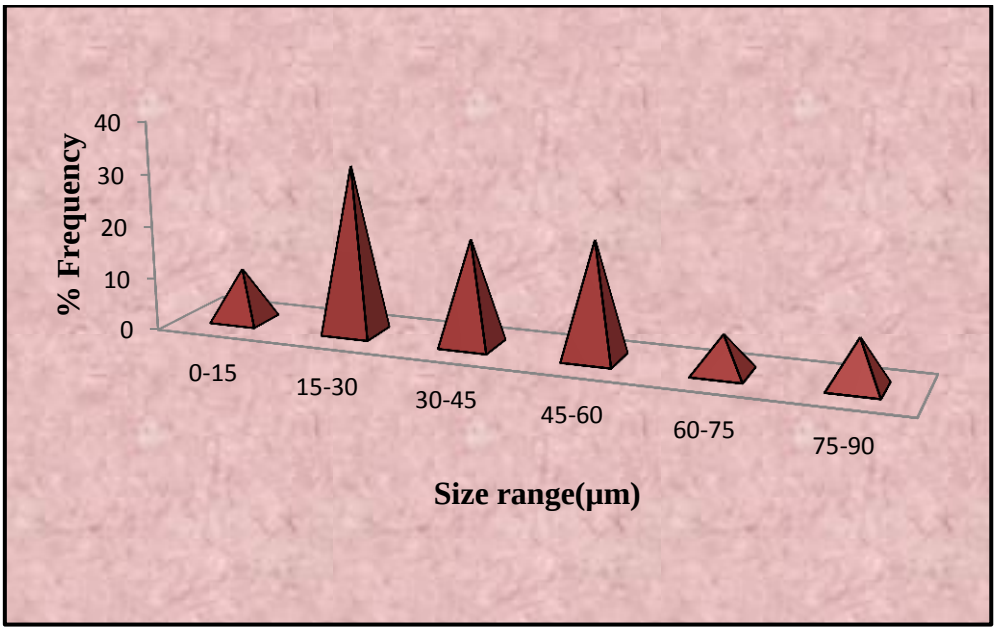


Fig. No. 9.13 : Particle size distribution of F1

Table No. 9.12 : Particle size distribution of formulation F1

Size range (µm)	Mean size (d)	No. of particles(n)	nd	% Frequency
0-15	7.5	10	75	10
15-30	22.5	32	720	32
30-45	37.5	20	750	20
45-60	52.5	22	1155	22
60-75	67.5	7	472.5	7
75-90	82.5	9	742.5	9

Σn = 100 Σnd = 3915

Σnd/Σn= 3915/100= 39.15µm

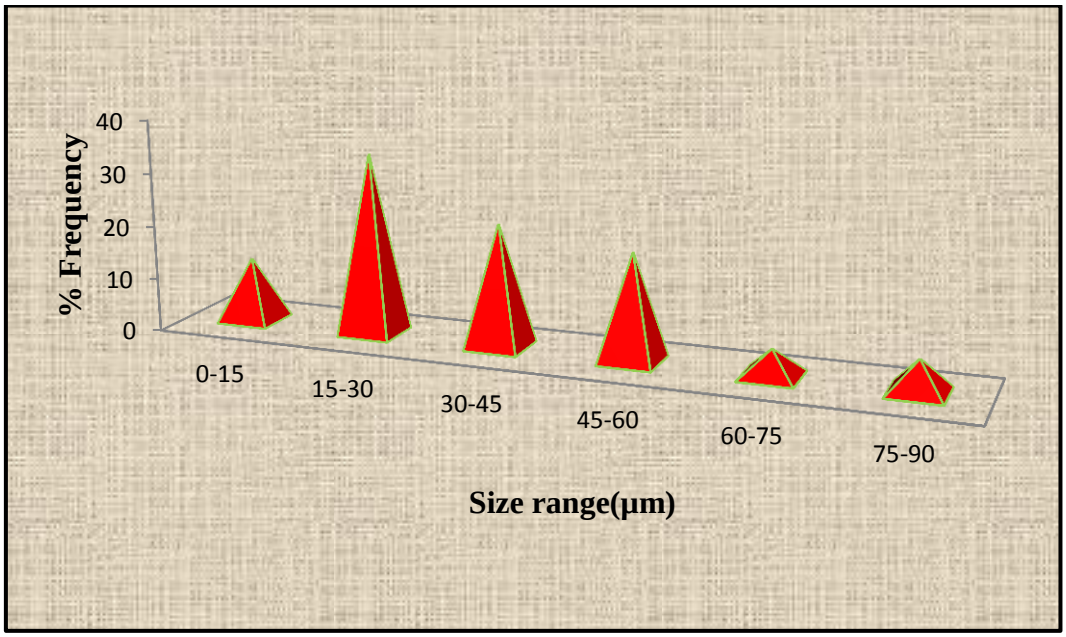


Fig. No. 9.14: Particle size distribution of F2

Table No.9.13: Particle size distribution of F2

Size range (µm)	Mean size (d)	No. of particles (n)	nd	% frequency
0-15	7.5	12	90	12
15-30	22.5	34	765	34
30-45	37.5	23	862.5	23
45-60	52.5	20	1050	20
60-75	67.5	5	337.5	5
75-90	82.5	6	495	6
		Σn = 100	**Σnd = 3600**	

Σnd/Σn= 3600/100= 36µm

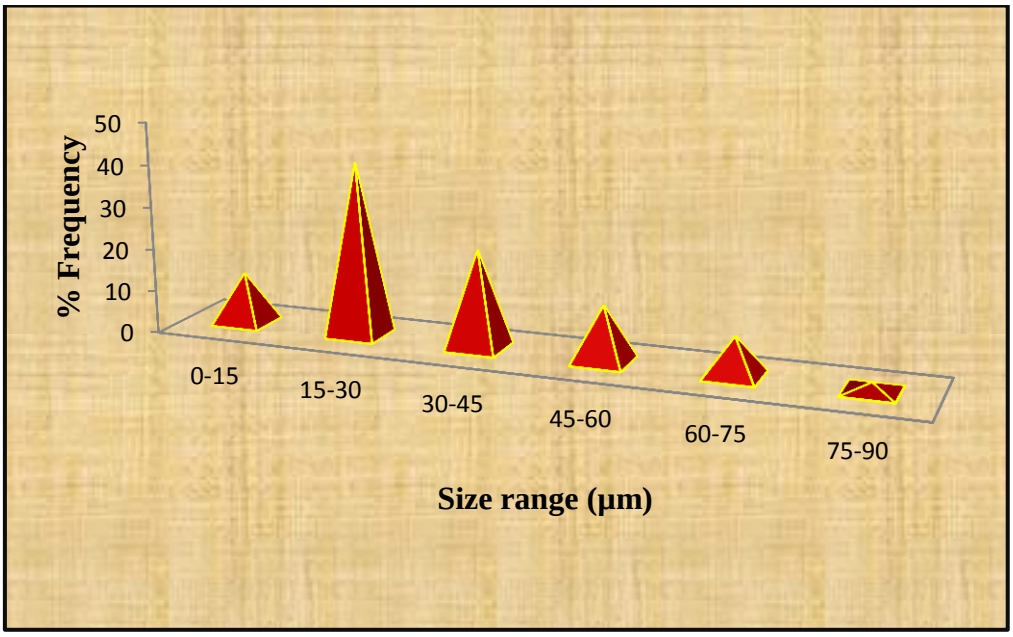


Fig. No. 9.15 : Particle size distribution of F3

Table No.9.14 Particle size distribution of F3

Size range (µm)	Mean size (d)	No. of particles (n)	nd	% Frequency
0-15	7.5	12	90	12
15-30	22.5	41	922.5	41
30-45	37.5	23	862.5	23
45-60	52.5	13	682.5	13
60-75	67.5	9	607.5	9
75-90	82.5	2	165	2
		Σn=100	**Σnd = 3330**	

Σnd/Σn= 3330/100= 33.30µm

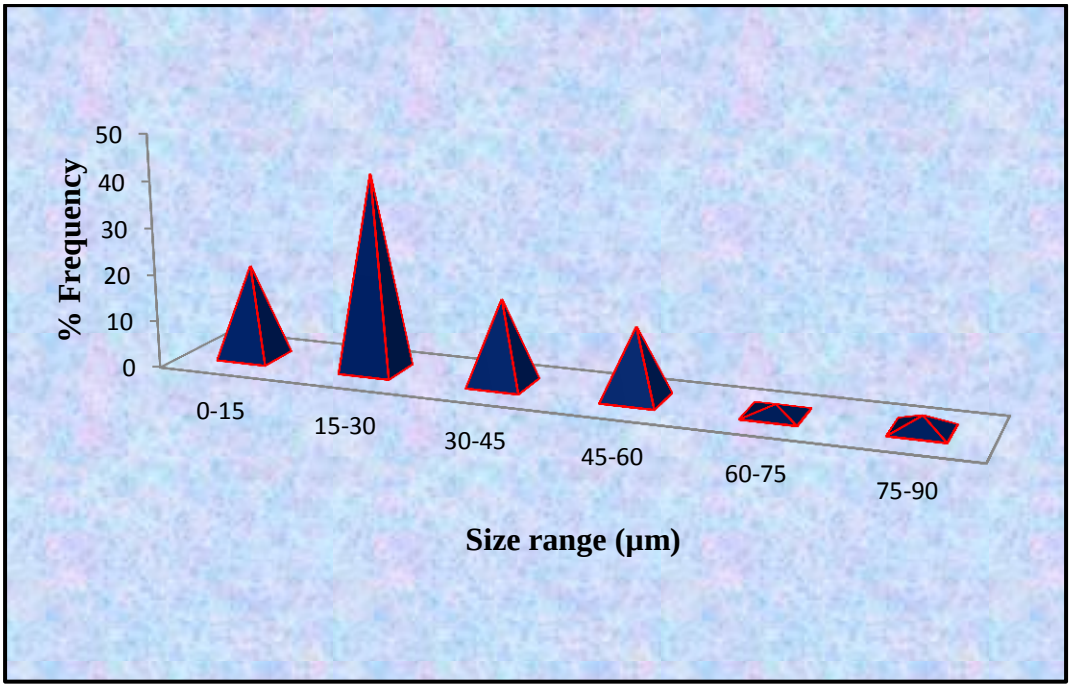


Fig. No.9.16: Particle size distribution of F4

Table No.9.15: Particle size distribution of F4

Size range (µm)	Mean size (d)	No. of particles (n)	nd	% Frequency
0-15	7.5	20	150	20
15-30	22.5	42	945	42
30-45	37.5	18	675	18
45-60	52.5	15	787.5	15
60-75	67.5	2	135	2
75-90	82.5	3	247.5	3
		Σn=100	**Σnd=2940**	

Σnd/Σn= 2940/100= 29.40µm.

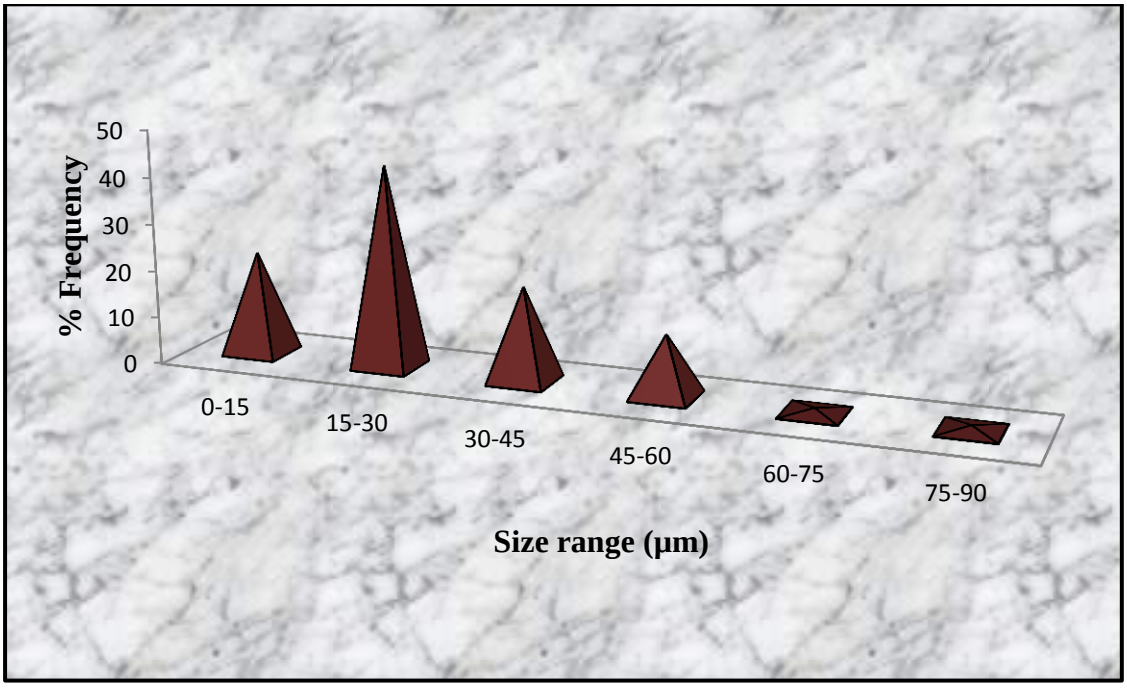


Fig. No.9.17: Particle size distribution of F5

Table No. 9.16: Particle size distribution of F5

Size range (in µm)	Mean diameter (d)	No. of particles (n)	nd	% Frequency
0-15	7.5	22	165	22
15-30	22.5	43	967.5	43
30-45	37.5	20	750	20
45-60	52.5	13	682.5	13
60-75	67.5	1	67.5	1
75-90	82.5	1	82.5	1
		Σn=100	**Σnd= 2715**	

Σnd/Σn= 2715/100= 27.15µm

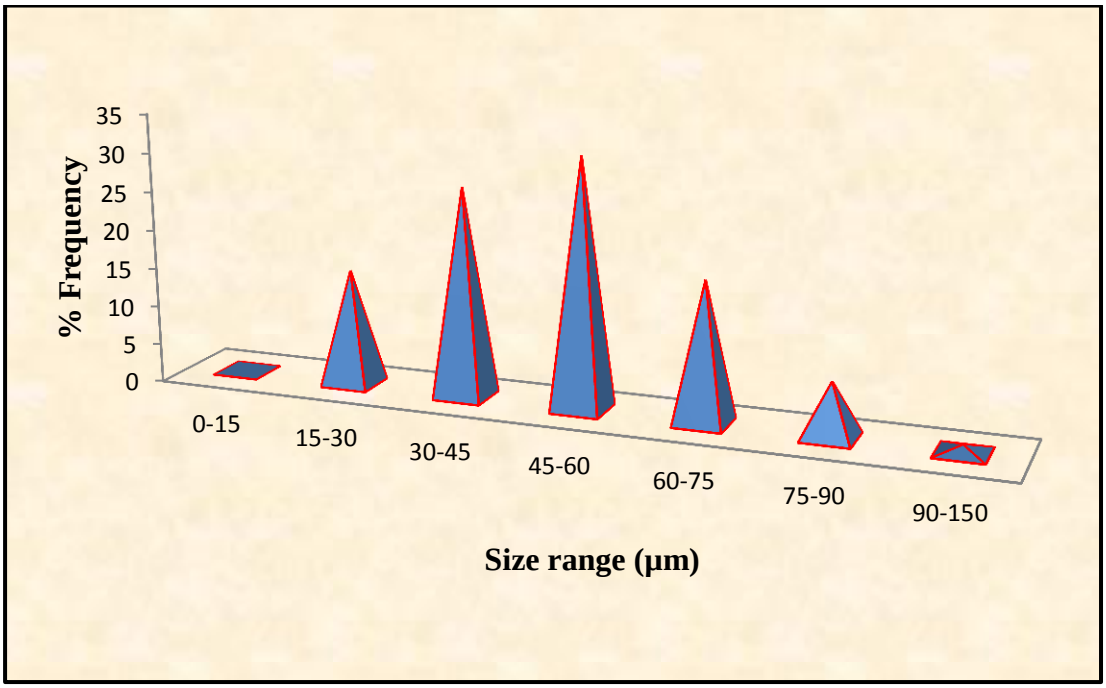


Fig. No. 9.18: Particle size distribution of F6

Table No.9.17: Particle size distribution of F6

Size range (µm)	Mean diameter (d)	No. of particles (n)	nd	% Frequency
0-15	7.5	0	0	0
15-30	22.5	15	337.5	15
30-45	37.5	27	1012.5	27
45-60	52.5	32	1680	32
60-75	67.5	18	1215	18
75-90	82.5	7	577.5	7
90-105	97.5	1	97.5	1
		Σn=100	**Σnd=4920**	

Σnd/Σn= 4920/100= 49.20µm

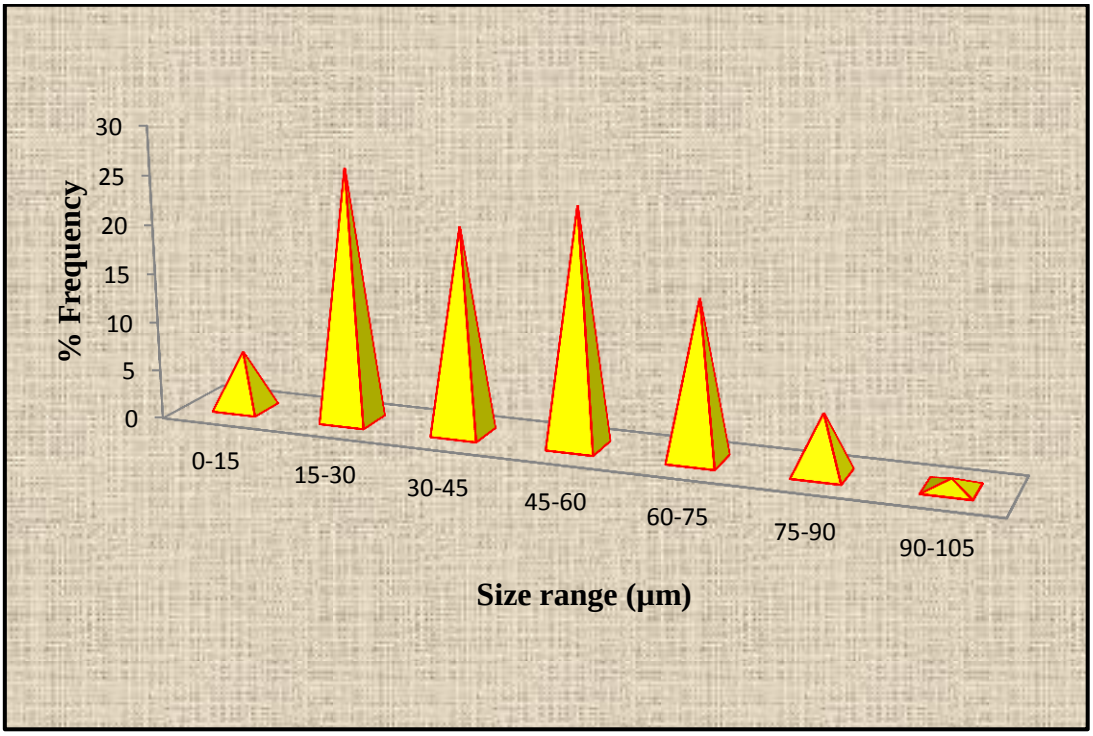


Fig. No. 9.19: Particle size distribution of F7

Table No.9.18: Particle size distribution of F7

Size range (µm)	Mean diameter (d)	No. of particles (n)	nd	% Frequency
0-15	7.5	6	45	6
15-30	22.5	26	585	26
30-45	37.5	21	787.5	21
45-60	52.5	24	1260	24
60-75	67.5	16	1080	16
75-90	82.5	6	495	6
90-105	97.5	1	97.5	1
		Σn=100	**Σnd=4350**	

Σnd/Σn=4350/100= 43.50µm

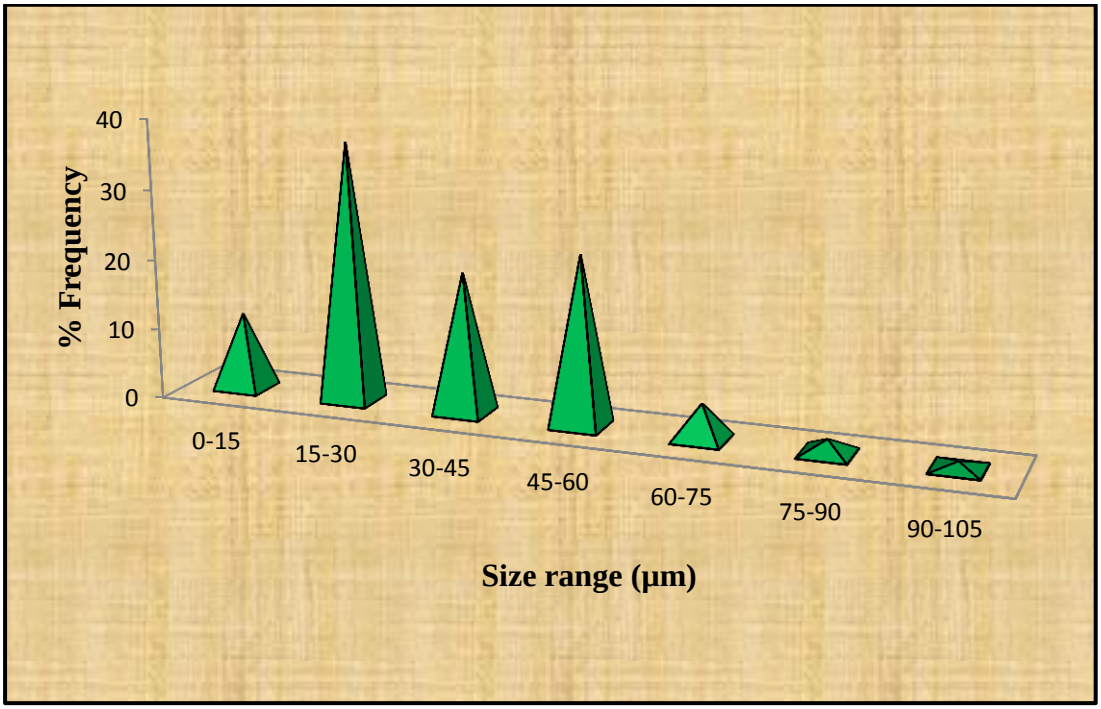


Fig. No.9.20: Particle size distribution of F8

Table No. 9.19: Particle size distribution of F8

Size range (µm)	Mean diameter (d)	No. of particles (n)	nd	% Frequency
0-15	7.5	11	82.5	11
15-30	22.5	37	832.5	37
30-45	37.5	20	750	20
45-60	52.5	24	1260	24
60-75	67.5	5	337.5	5
75-90	82.5	2	165	2
90-105	97.5	1	97.5	1
		Σn=100	**Σnd=3525**	

Σnd/Σn=3525/100= 35.25µm

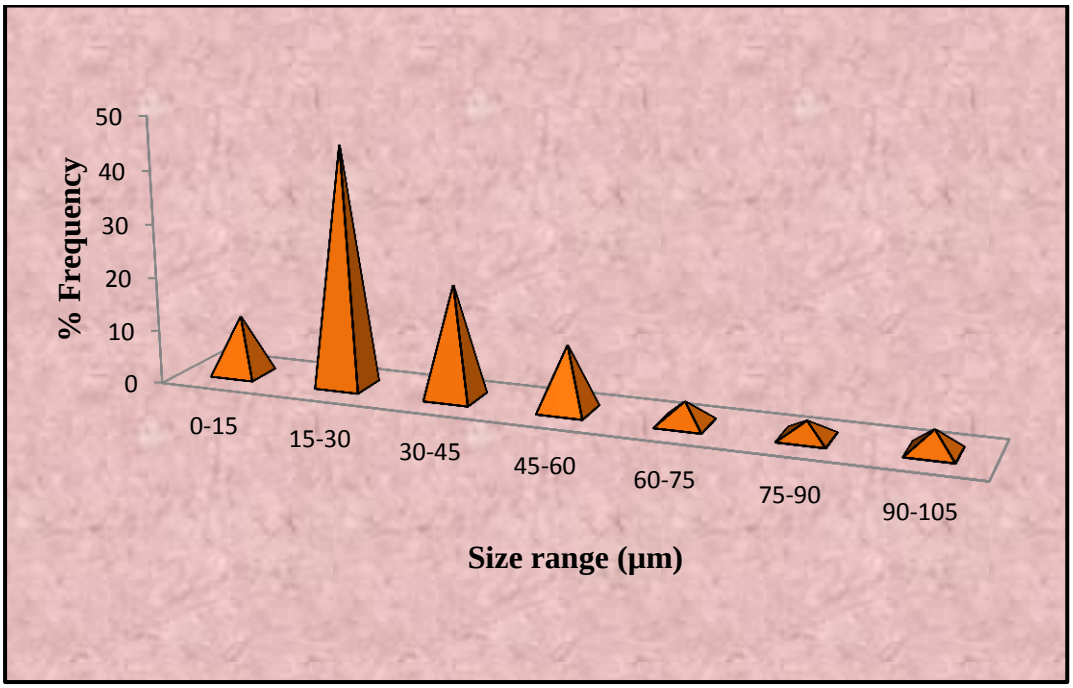


Fig. No.9.21: Particle size distribution of F9

Table No. 9.20: Particle size distribution of F9

Size range (µm)	Mean diameter (d)	No. of particles (n)	nd	% Frequency
0-15	7.5	11	82.5	11
15-30	22.5	45	1012.5	45
30-45	37.5	21	787.5	21
45-60	52.5	12	630	12
60-75	67.5	4	270	4
75-90	82.5	3	247.5	3
90-105	97.5	4	390	4
		Σn=100	**Σnd=3420**	

Σnd/Σn=3420/100= 34.20µm

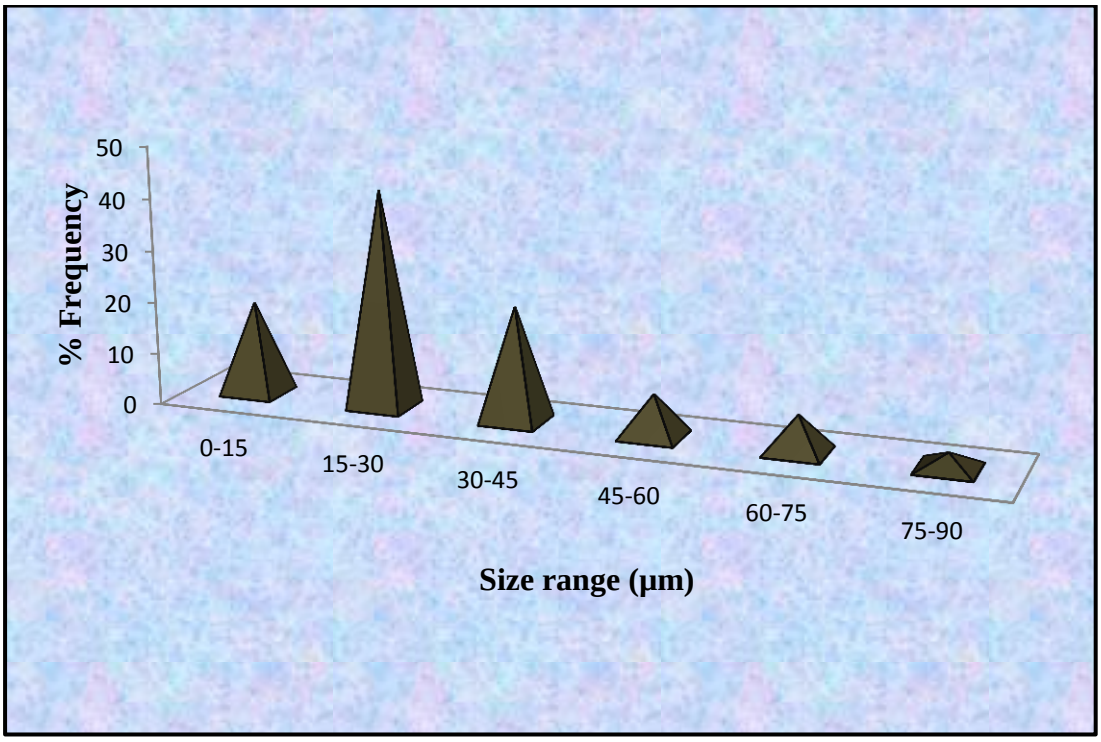


Fig. No. 9.22: Particle size distribution of F10

Table No. 9.21: Particle size distribution of F10

Size range (µm)	Mean diameter (d)	No. of particles (n)	nd	% frequency
0-15	7.5	18	13.5	18
15-30	22.5	42	945	42
30-45	37.5	22	825	22
45-60	52.5	8	420	8
60-75	67.5	7	472.5	7
75-90	82.5	3	247.5	3
		Σn=100	**Σnd=3045**	

Σnd/Σn= 3045/100= 30.45µm

AVERAGE PARTICLE SIZE OF MICROSPONGES

Table No. 9.22: Average particle size of microsponges

S.No.	Formulations	Average particle size (µm)
1	F1	39.15
2	F2	36.00
3	F3	33.30
4	F4	29.40
5	F5	27.15
6	F6	49.20
7	F7	43.50
8	F8	35.25
9	F9	34.20
10	F10	30.45

From the above results, it was found that particle size distribution was in the range of 27.15µm to 43.50µm. This reveals as drug: polymer increases the particle size went on decreasing.The results corresponds to earlier reports[39]

***In-vitro* drug release of microsponges**

The *in-vitro* release of various formulation are shown in Table No. 9.23

Table No. 9.23: *In-vitro* drug release of microsponges

Time (Hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	05.54	05.90	06.08	06.80	06.78	03.94	05.00	05.54	06.78	06.78
2	15.00	15.71	16.43	16.95	17.49	09.12	10.37	11.08	11.44	12.69
3	23.44	24.68	25.23	26.29	27.72	12.72	16.84	21.81	23.24	24.67
4	31.76	32.47	33.72	34.27	36.07	25.25	25.82	26.56	27.12	27.83
5	40.10	41.36	44.40	44.59	45.50	28.95	30.23	30.97	33.13	33.85
6	46.19	51.38	52.48	53.55	53.16	36.22	38.05	41.82	43.63	44.89
7	54.57	56.63	57.03	57.77	58.14	45.13	45.71	46.67	46.71	48.87
8	54.57	59.29	59.82	59.85	60.60	55.51	56.11	56.20	57.46	57.56
9	64.64	67.06	65.50	67.30	70.18	64.89	65.65	66.98	68.27	67.78
10	74.42	75.07	76.34	76.90	78.21	75.02	76.16	77.84	79.14	79.36
11	78.39	79.02	80.30	80.88	82.72	79.53	80.48	81.63	82.05	84.58
12	83.78	87.38	88.75	94.11	98.60	83.33	83.94	86.34	97.88	89.66

‘

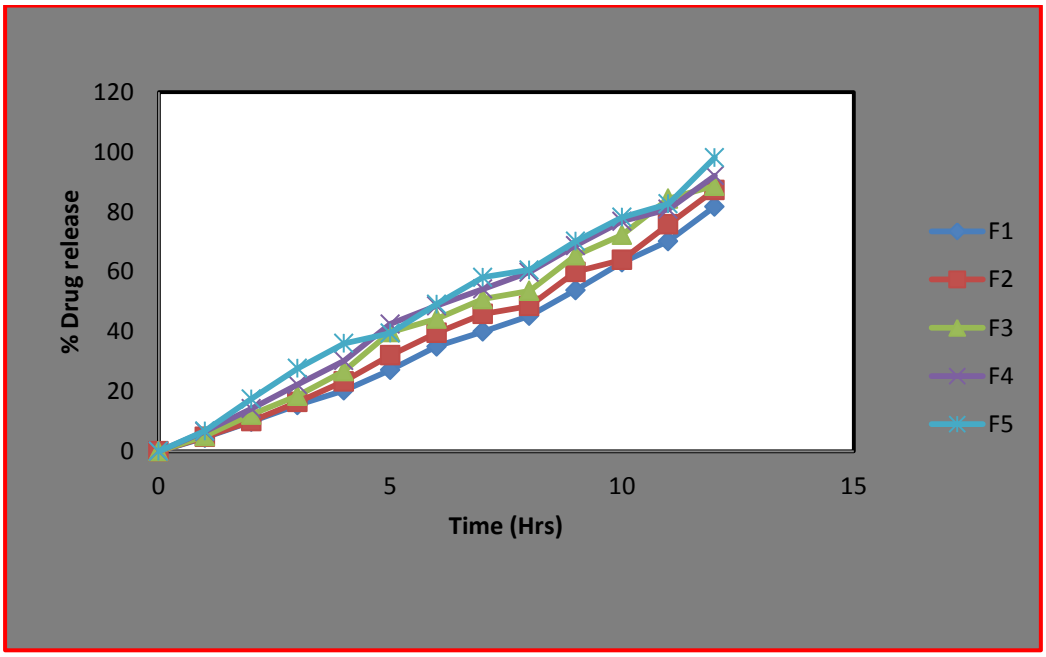


Fig. No. 9.23: *In-vitro* release study of microsponges (F1-F5)

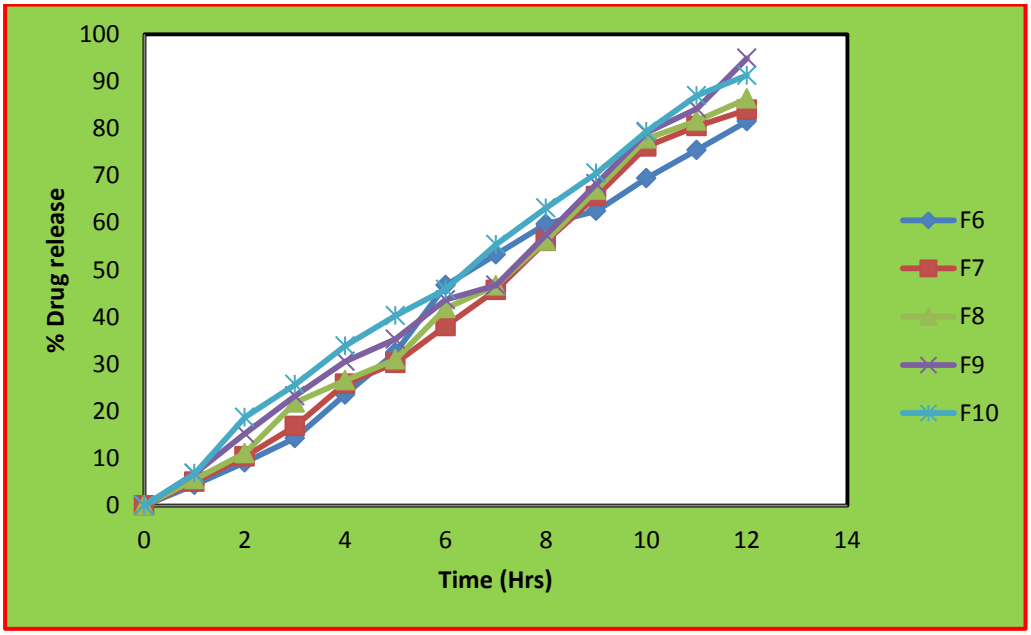


Fig. No. 9.24: *In-vitro* release study of microsponges (F6-F10)

***In-vitro* drug release study of optimized formulation (F5,F9)**

Table No.9.24: *In-vitro* release of optimized formulation

Time(in Hrs)	% Drug release		
	F5	F9	Marketed formulation
1	6.9±0.30	6.36±0.71	42.74±0.05
2	16.31±1.20	10.49±0.73	100.23±0.16
3	25.7±1.75	21.87±1.68	-
4	35.39±0.51	26.86±0.22	-
5	46.02±0.62	32.45±0.95	-
6	52.53±0.49	43.01±0.51	-
7	57.70±2.27	45.80±0.67	-
8	61.69±1.85	56.84±0.51	-
9	69.80±1.21	67.34±0.74	-
10	76.41±1.38	78.50±0.45	-
11	85.89±5.32	84.2±3.21	-
12	98.3±0.21	97.88±1.14	-

*Mean±SD (n=3)

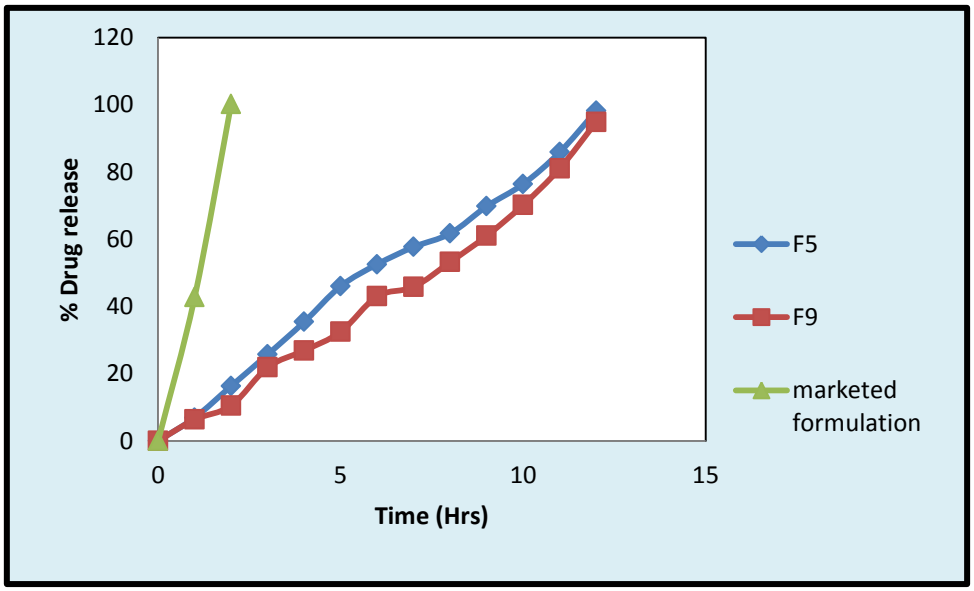


Fig. No. 9.25: *In-vitro* drug release study of optimized formulation

EVALUATION OF OPTIMIZED FORMULATIONS

EFFECT OF STIRRING RATE

The effect of stirring rate of F5 and F9 formulations was studied on % yield, mean particle size, % drug content & % drug release. The results are listed in table No.9.25

Table No.9.25: Stirring rate effect on F5 and F9

F. Code	Internal phase composition			External phase composition		Stirring rate (rpm)	% yield	Mean Particle Size (µm)	% Drug content (% w/w)	% Drug release
	Drug (g)	Polymer (g)	Solvent (ml)	Water (ml)	PVA(g)					
F5	1	0.2	5	100	0.2	800	88.09	35.25	90.07	95.12
	1	0.2	5	100	0.2	1000	84.02	27.15	95.15	98.10
	1	0.2	5	100	0.2	1200	82.61	22.95	98.04	97.25
F9	0.8	0.2	5	100	0.2	800	85.00	41.55	87.29	97.88
	0.8	0.2	5	100	0.2	1000	77.87	34.20	90.44	91.61
	0.8	0.2	5	100	0.2	1200	70.81	26.25	94.38	93.11

The results showed increasing stirring speed from 800 to 1200 rpm for both optimized formulations revealed decrease in % yield due to turbulence created within the external phase, polymer then adhered to the stirrer. Upon increasing stirring speed reduction of mean particle size was observed as a vigorous, uniform, increased mechanical shear is imposed and this resulted in rapid dispersion of formed droplets which may have less chance of coalescing into bigger droplets. Drug content was increased on increasing stirring rate. The results corresponds to earlier reports. [37,14,20] Drug release showed no particular pattern.

EFFECT OF CONCENTRATION OF EXTERNAL PHASE

The effect of external phase of F5 and F9 formulations was studied on % yield, mean particle size, % drug content & % drug release. The results are listed in table No.9.26

Table No.9.26: Effect of amount of External phase on F5 and F9

F. Code	Internal phase composition			External phase composition		Stirring rate (rpm)	% yield	Mean Particle Size (µm)	% Drug Content (% w/w)	%Drug release
	Drug (g)	Polymer (g)	Solvent (ml)	Water (ml)	PVA (g)					
F5	1	0.2	5	100	0.2	1000	84.02	27.15	95.15	98.10
	1	0.2	5	100	0.3	1000	82.01	28.80	93.47	89.84
	1	0.2	5	100	0.4	1000	81.43	33.30	90.57	93.69
F9	0.8	0.2	5	100	0.2	1000	77.87	34.20	90.44	97.88
	0.8	0.2	5	100	0.3	1000	69.23	39.15	88.54	89.68
	0.8	0.2	5	100	0.4	1000	65.68	43.50	82.74	94.01

It was observed that on increasing concentration of emulsifying agent on both formulations there was an increase in viscosity which resulted in larger emulsion droplets and finally in greater microsponges size. When the concentration of emulsifier was increased, the production yield and drug content decreased.[16] Drug release showed no particular pattern.

EFFECT OF CONCENTRATION OF INTERNAL PHASE

The effect of internal phase of F5 and F9 formulations was studied on % yield, mean particle size, % drug content & % drug release. The results are listed in table No.9.27

Table No.9.27: Effect of concentration of Internal phase on F5 and F9

F. Code	Internal phase composition			External phase composition		Stirring rate (rpm)	% yield	Mean particle size (µm)	% Drug content (% w/w)	% Drug release
	Drug (g)	Polymer (g)	Solvent (ml)	Water (ml)	PVA (g)					
F5	1	0.2	5	100	0.2	1000	84.02	29.85	95.15	98.10
	1	0.2	10	100	0.2	1000	82.12	27.15	91.04	90.31
	1	0.2	15	100	0.2	1000	79.44	24.15	85.51	93.02
F9	0.8	0.2	5	100	0.2	1000	87.87	34.20	90.44	97.88
	0.8	0.2	10	100	0.2	1000	82.16	28.20	88.60	87.14
	0.8	0.2	15	100	0.2	1000	74.66	25.34	85.47	92.38

It was found that by increasing the solvent volume the particle size of microsponges decreases. The particle size of microsponges were directly proportional to the viscosity of the dispersed phase. When the dispersed phase with higher viscosity was poured into the external phase, due to the higher viscosity of the internal phase, the globules of the formed emulsion could not be divided into smaller particles and bigger droplets were formed and mean particle size increased. When the amount of solvent was increased, drug content of microsponges decreased. This was probably due to the lower concentration of the drug in the high volume of solvent..[20,37] There was no particular pattern followed for drug release.

OPTIMISATION

Even on increasing drug: polymer ratio, amount of solvent, concentration of emulsifying agent and rate of stirring on optimized microsponges (F5, F9). F5 was optimised as the best formulation as it showed better results.

***In-vitro* anti-inflammatory activity**

Table No. 9.28: *In-vitro* anti-inflammatory activity of F5

Time (Hrs)	% Inhibition	
	Pure drug	F5
0.5	46.04	-
1	68.35	30.93
1.5	83.87	-
2	-	37.01
3	-	39.65
4	-	45.94
5	-	50.60
6	-	53.54
7	-	60.14
8	-	64.60
9	-	70.68
10	-	74.34
11	-	81.54
12	-	83.77

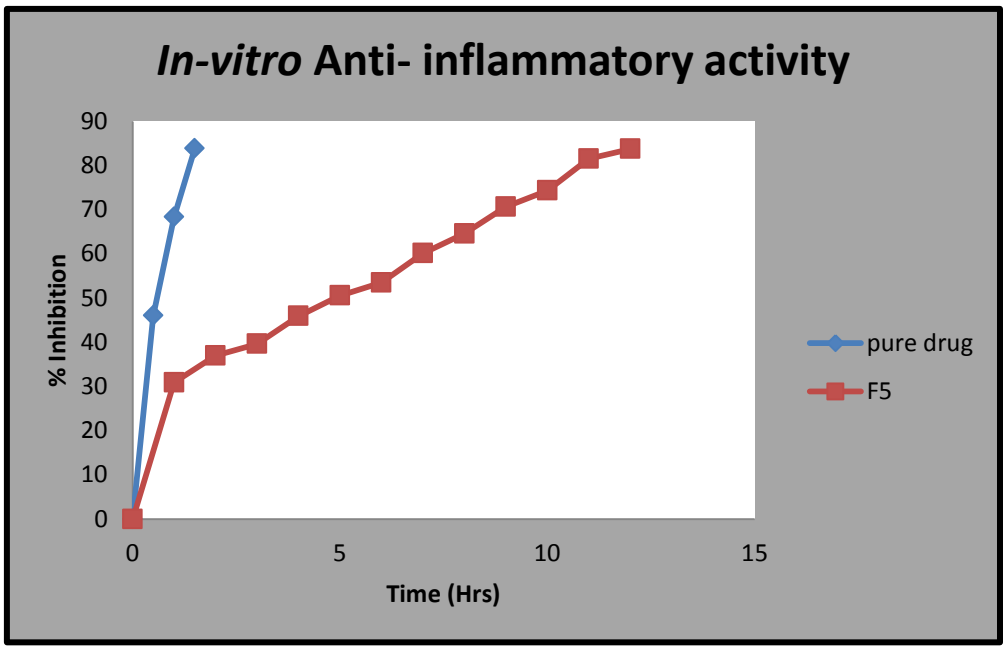


Fig No. 9.26 : *In-vitro* anti-inflammatory activity

Results of *in-vitro* anti-inflammatory activity by albumin denaturation method showed that the optimized formulation F5 inhibited approximately 80% within 12 hrs. F5 also exhibits a satisfactory dose dependent anti-inflammatory activity.[76]

PREFORMULATION STUDIES OF THE DRUG AND OPTIMIZED FORMULATION

Table No.9.29: Preformulation parameters of drug and microsponges*

F. Code	Bulk density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Hausner's ratio	Angle of repose(θ)	Porosity (%)
Drug	0.299±0.002	0.478±0.001	37.44±0.002	1.564± 0.08	$48^{o}57'$± 0.71	51
F5	0.394±0.01	0.454±0.00	13.03±3.34	1.151±0.04	$30^{o}03'$±1.61	73

*Mean±SD (n=03)

The flow property of pure drug was found to be very poor. Good flow property was observed for microsponges.[40]

PREPARATION OF CAPSULE

The optimised microsponges were filled into "1" size capsule each containing 100mg equivalent of DCN.

POST FORMULATION STUDIES

1.Uniformity of weight*

Table No. 9.30: Uniformity of weight of Optimized capsule

Formulation	Average weight of Capsule (g)
F5	0.154±0.0002

*Mean±SD (n=20)

The capsules comply with the official test for Uniformity of weight.[76]

2.Disintegration test

Table No. 9.31: Disintegration test of Empty capsule

Capsule	Time (Min)
Empty capsule	11

The capsules comply with the official test for Disintegration test.[76]

3. Drug content*

The contents of active ingredients in various formulations are given in the Table No.

Table No. 9.32: Drug content of optimized formulation

Formulation	Drug content
F5	98.65±0.804

*Mean±SD (n=5)

The drug content was within limits i.e was not less than 90% and not more than 110%.(as per IP 2014).[76]

4. *In- vitro* drug release study of F5 Capsule*

Table No.: 9.33 *In-vitro* drug release study of Capsule.

S.No.	Time(Hrs)	% drug release
1	1	6.87±0.09
2	2	15.63±0.62
3	3	24.69±0.18
4	4	35.23±0.09
5	5	45.74±0.61
6	6	52.72±0.59
7	7	57.30±0.80
8	8	62.69±0.53
9	9	75.79±0.36
10	10	82.42±0.26
11	11	94.76±0.80
12	12	98.16±0.26

* Mean±SD (n=3)

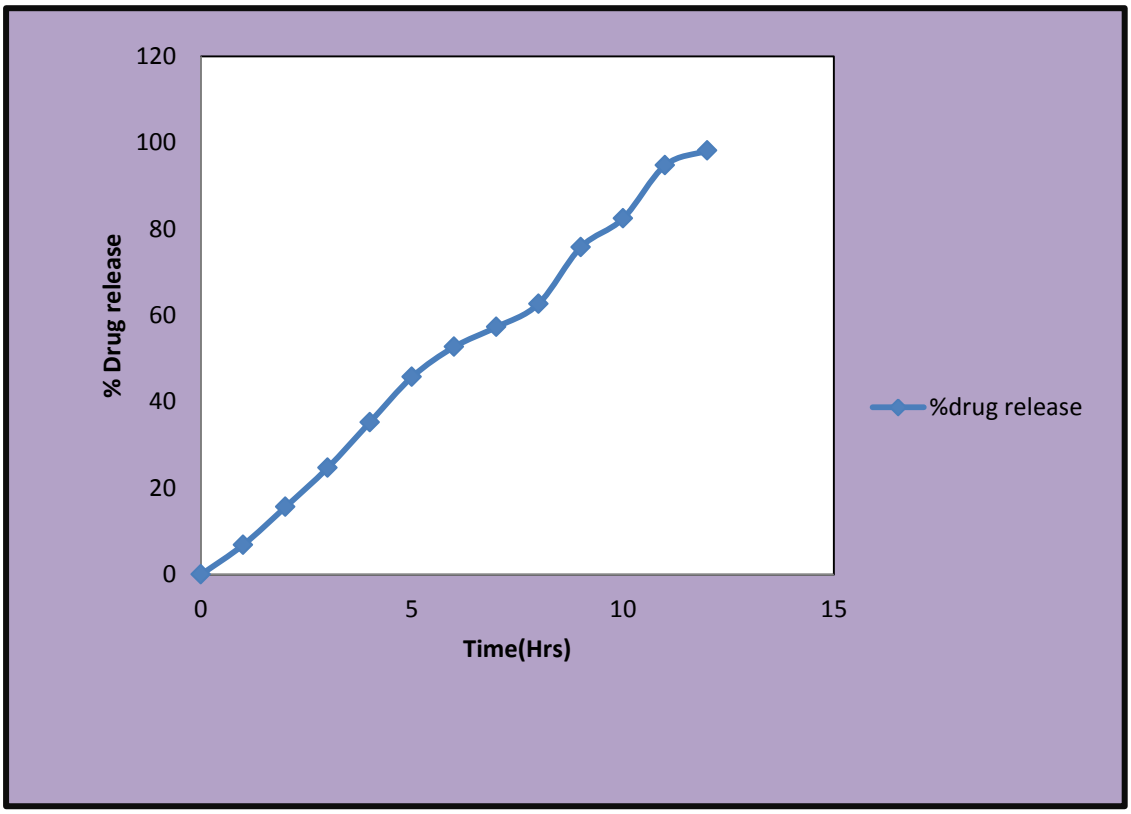


Fig. No.9.27: *In-vitro* release study of F5 capsule

RELEASE KINETICS OF THE OPTIMIZED FORMULATION

Table No. 9.34: Release kinetics of the optimised formulation (F5)

Time (Hrs)	Log time (Hrs)	Sq. Root of time (Hrs)	Cum % drug release	Cum % drug remaining	Log cum % drug release	Log cum % drug remaining	Cube root of cum % drug remaining
0	-∞	0	0	100	-∞	2	4.64
1	0	1	6.87	93.13	0.83	1.96	4.53
2	0.30	1.41	15.63	84.37	1.19	1.92	4.38
3	0.47	1.73	24.69	75.31	1.39	1.87	4.22
4	0.60	2	35.23	64.77	1.54	1.81	4.01
5	0.69	2.23	45.74	54.26	1.66	1.73	3.78
6	0.77	2.44	52.72	47.28	1.72	1.67	3.61
7	0.84	2.64	57.3	42.7	1.75	1.63	3.49
8	0.90	2.82	62.69	37.31	1.79	1.57	3.34
9	0.95	3	69.74	30.26	1.84	1.48	3.11
10	1	3.16	75.42	24.58	1.87	1.39	2.90
11	1.04	3.31	90.76	9.24	1.95	0.96	2.09
12	1.07	3.46	98.16	1.84	1.99	0.26	1.22

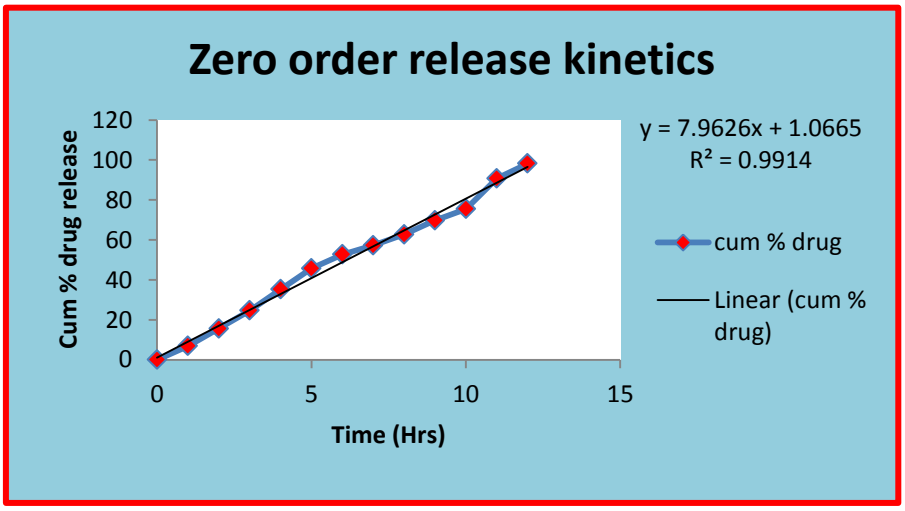


Fig. No. 9.28: A Plot of Zero order kinetics

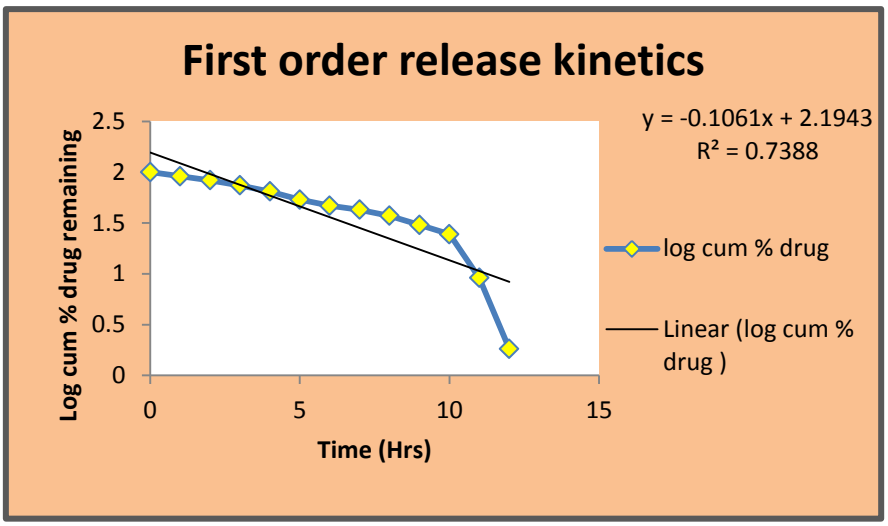


Fig. No.9.29: A Plot of First order kinetics

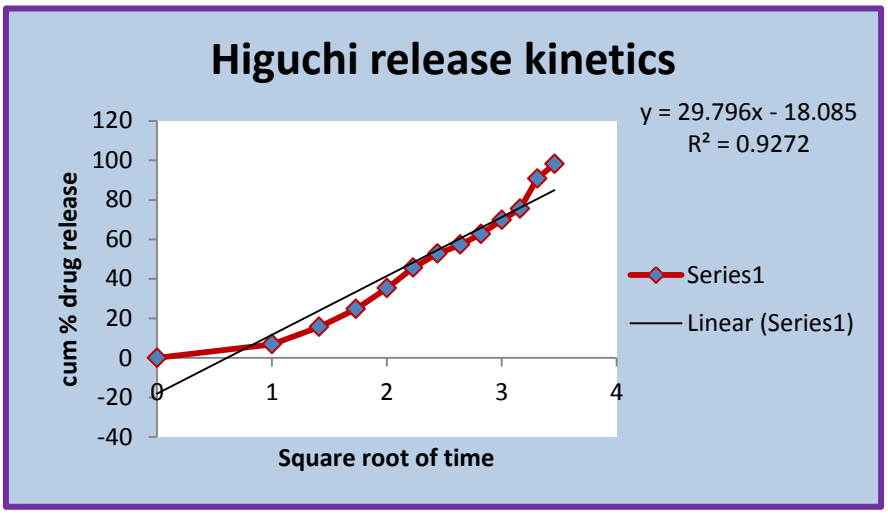


Fig. No. 9.30: A Plot of Higuchi release kinetics

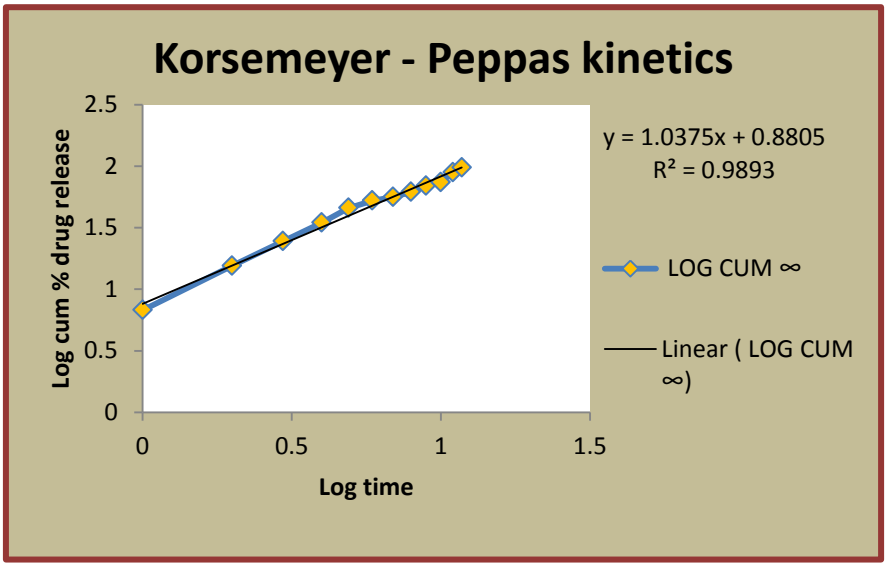


Fig. No. 9.31: A Plot of Korsemeyer- Peppas kinetics

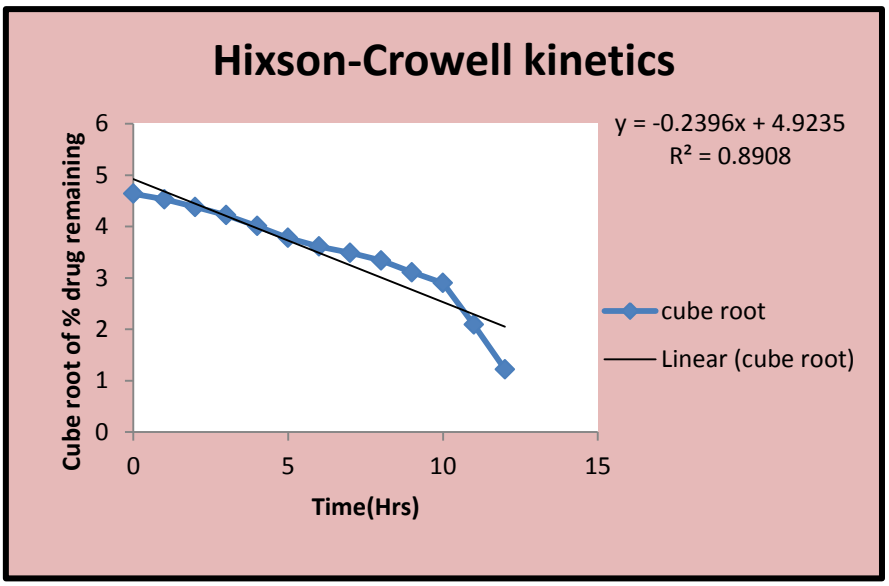


Fig. No. 9.32: A Plot of Hixson- Crowell kinetics

The coefficient of determination (R^2) was taken as criteria for choosing the most appropriate model. The R^2 values of various models are in table No.9.35

Table No. 9.35: R^2 values of various kinetics models

Kinetics models	Coefficient of determination (R^2)
Zero order	0.991
First order	0.738
Higuchi	0.927
Korsemeyer and Peppas	0.989
Hixson Crowell	0.890

The *in-vitro* drug release of the optimized formulation F5 was best explained by Zero order kinetics as the plots showed linearity (R^2=0.991). This Zero order kinetics explains the controlled release of the prepared microsponges over the period of time.[23]

SUMMARY AND CONCLUSION

Diacerein is a poorly soluble drug with short half life, thus selected as a model drug for MDDS to overcome these problems and to release the drug in a controlled manner. Diacerein is formulated as Microsponges by Quasi emulsion solvent diffusion method using polymers Eudragit RS 100 and Ethyl cellulose and finally enclosed in Capsules.

- ✓ Compatibility studies were performed for drug and excipients
- ✓ Physical compatibility study showed drug and excipients were physically compatible with each other.
- ✓ Chemical compatibility study (FT-IR) was carried out. It revealed no interaction between the drug and excipients.
- ✓ Standard graph was drawn for Diacerein and it was found that the solutions showed linearity (R^2=0.999) and obeyed Beer Lambert's law.
- ✓ Diacerein Microsponges were prepared using two polymers to determine which polymer retards the release better.
- ✓ The *in-vitro* release was carried out for all the formulations. The formulation F5 (containing 5:1 drug: polymer (Eudragit RS 100) ratio) released 98.30% and F9 (containing 4:1 drug: polymer(Ethyl cellulose) ratio)at the end of 12^{th} hours. Therefore F5 and F9 were selected as optimized formulations.
- ✓ The effect of stirring rate was studied on optimized formulations for determining production yield, drug content, mean particle diameter and drug release. The stirring rate increases production yield and drug content, while mean particle diameter decreased. No particular pattern was observed for drug release.
- ✓ The effect of internal phase concentration was studied on optimized formulations for determining production yield, drug content, mean particle diameter and drug release. The internal phase concentration increases production yield, drug content and decreases mean particle diameter. F5 showed a decrease in drug release with increase in solvent amount, but F9 showed no particular pattern.

- ✓ The effect of external phase concentration was studied on optimized formulations for determining production yield, drug content, mean particle diameter. The external phase concentration increases production yield, mean particle diameter but decreases drug content. No particular pattern was followed for drug release.
- ✓ *In-vitro* anti-inflammatory activity by albumin denaturation method showed that the optimized formulation F5 inhibited approximately 80% within 12 hrs which clearly indicates that F5 also has exhibits a satisfactory dose dependent anti-inflammatory activity.
- ✓ Even on increasing drug: polymer ratio, amount of solvent, concentration of emulsifying agent and rate of stirring on optimized microsponges (F5, F9). F5 was optimised as the best formulation as it showed better results.
- ✓ Preformulation study was carried out for drug and F5 microsponges. It revealed that the flow property of pure drug was very poor, but the microsponges has good flow.
- ✓ Post formulation parameters of capsules were evaluated and the results were found to comply with the official specifications.
- ✓ The dissolution data of the optimized formulation were fitted to various kinetic models and the formulation F5 fitted best to Zero order kinetics.

It is concluded that F5 formulation containing 5:1 drug:polymer ratio with Eudragit RS 100 produced Controlled release.

Future scope

1. *In-vivo* study.
2. Pharmacokinetic and toxicity study.
3. Stability studies.

1. Brahmankar DM and Sunil B Jaiswal. Biopharmaceutics and Pharmcokinectics – A Treatise. Second edn. New Delhi: Vallabh Prakashan; 2009; 399-400.

2. Vyas SP and Khar RK. Controlled drug delivery- Concepts and advances. Delhi: Vallabh Prakashan, 2002, 155-157.

3. Namrata Jadhav, Vruti Patel, Siddesh Mungekar, Gaurav Bhamare, Manisha Karpe, Vilasrao Kadams. Microsponge Delivery System: An updated review, current status and future prospects. Journal of Scientific and Innovative Research. 2013; 2 (6): 1097-1110.

4. Sandhyarani Sagavkar R, Shrinivas Mohite K. Innovative And Novel Strategy: Microsponges Drug Delivery System . International Journal of Universal Pharmacy and Bio Sciences 2014; 3(4):79-92.

5. Charde MS, Ghanawat PB, Welankiwar AS, Kumar J, Chakole RD. Microsponge A Novel New Drug Delivery System: A Review. International Journal of Advances in Pharmaceutics.2013; 2 (6) :63-70.

6. Kapoor D, Vyas RB, Lad C, Patel M, Tyagi BL. A Review On Microsponge Drug Delivery System . Journal of Drug Delivery & Therapeutics. 2014; 4(5): 29-35

7. Shyam Sunder Mandava and Vedavathi Thavva. Novel Approach: Microsponge Drug Delivery System. International Journal of Pharmaceutical Science and Research, 2012; 3(4): 967-980.

8. Yerram Chandramouli , Shaik Firoz, Rubia Yasmeen, Amaravathi Vikram, Mahitha B, Aruna U. Microsponges: A Novel Drug Delivery System For Controlled Delivery Of Topical Drugs. International Journal of Pharmaceutical Research & Analysis . 2010; 2 (2): 79-86

9. Manisha K Tile and AY Pawar. Microsponges: A novel strategy for Drug delivery.International Journal of Pure and Applied Biosciences.2015; 3 (1): 224-235.

10. Hibah Aldawsari and Shaimaa Badr-Eldin .Microsponges as promising vehicle for drug delivery and targeting: Preparation, characterization and applications. African Journal of Pharmacy and Pharmacology.2014; 7(17): 873-881.

11. Saurabh Kumar, LK Tyagi and Dashrath Singh. Microsponge Delivery System (MDS): A Unique Technology For Delivery Of Active Ingredients . International Journal of Pharmaceutical Science and Research. 2011; Vol. 2(12): 3069-3080.

12. Ravi R, SK Senthilkumar, S Parthiban .Microsponges Drug Delivery System: A Review. International Journal of Pharmacy Review & Research. 2013; 3(1):6-11.

13. Hamid Hussain, Divya Juyal, Archana Dhyani . Microsponges: An Overview . International Journal of Drug Delivery Technology. 2014; 4(4): 58-66.

14. Viral Shaha, Hitesh Jain, Jethva Krishna, Pramit Patel. Microsponge drug delivery: A review. International Journal Research Pharmaceutical Science. 2010;1(2): 212-218.

15. Ankaj Kaundal, Rohit Bhatia, Amit Sharma, Pankaj Sukrial. A Review On Microsponges Drug Delivery System. International Journal of Advanced Pharmaceutics. 2014;4(3) :177-181.

16. Vikas Jain and Ranjith Singh. Development and characterization of Eudragit RS 100 loaded microsponges and its colonic delivery using natural polysaccharides. Acta Poloniae Pharmaceutica- Drug research .2010;67(4):407-415.

17. Vikas Jain and Ranjith Singh. Dicyclomine -loaded Eudragit – based Microsponge with Potential for Colonic Delivery:Preparation and Characterization. Tropical Journal of Pharmaceutical Research. 2010;9(1):67-72.

18. Anilkumar J,Shinde,Manoj B,Paithane, Sujata Sawant. Development and Evaluation of Fenoprofen Microsponges and its Colonic Delivery using

Natural Polysaccharides. American Journal of Pharmaceutical Sciences and Nanotechnology. 2014; 1(1) : 27-42.

19. Riyaz Ali M Osmani, Nagesh Aloor, Bharathi Thaware, Parthasarrathi Kulkarni. Microsponges based drug delivery system for augmented gastroparesis therapy: Formulation development and evaluation. 1-24.

20. Karthika R, Elango K, Ramesh Kumar K, Rahul K. Formulation and Evaluation of Lornoxicam Microsponge Tablets for the treatment of Arthrits. International Journal of Pharmaceutical Innovations. 2013; 3(2) : 29-40.

21. Sonali, Rahul Pratap Singh and Sunil Kumar Prajapati. Formulation and evaluation of Prednisolone loaded microsponges for colon drug delivery: *In-vitro* and Pharmacokinectic study. International Journal of Pharmaceutical Sciences and Research. 2014; 5(5) : 1994-2005.

22. Rashmi Sareen, Kavita Nath, Nitin Jain and KL Dhar. Curcumin Loaded Microsponges for Colon Targetin in Inflammatory Bowel Disease: Fabrication, Optimization, and *In-vitro* and Pharmacodynamic Evaluation. BioMed Research International. 2014:1-7.

23. Ramani Gade, Anitha Makineni, Aparna.A, Krishna Keerthi B, TEGK Murthy, Chandu Babu Rao, Sreekanth. Design and development of Hydroxyzine hydrochloride controlled release tablets based on Microsponge technology. Carib.J. Sci Tech. 2013; 1(1): 172-184.

24. Shah Harsh, Karishma Patel, UMU padhyay. Formulation and evaluation of controlled release colon targeted microsponge of Aceclofenac. The Pharma Innovation Journal. 2014; 3(10):81-87.

25. Riyaz Ali M Osmani, Nagesh Aloor, Bharathi Thaware, Parthasarrathi Kulkarni. Microsponges based novel drug delivery system for augmented Arthritis therapy. Saudi Pharmaceutical Journal. 2015: 1-11.

26. John I D'souza and Harinath N More.Topical anti- inflammatory gels of Fluocinolone Acetonide entrapped in Eudragit based microsponge delivery system. Research J Pharma and Tech. 2008; 1 (4) : 502-506.

27. Markand Mehta, Amish Panchal, Viral H Shah, Umesh Upadhyay. Formulation and *In- vitro* evaluation of controlled release microsponge gel for topical delivery of Clotrimazole. International Journal of Advanced Pharmaceutics. 2012; 2(2): 93-101.

28. Hamid Hussain, Archana Dhyani, Divya Juyal, Abhishek Bahuguna. Formulation and evaluation of gel- loaded microsponges of Diclofenac sodium for topical delivery. The Pharma Innovation Journal. 2014; 3(10): 58-63.

29. Makwana Rajeshree, Patel Harsha, Patel Vishnu. Photostability enhancement of Micanazole nitrate by microsponge formulation. International Journal of Current Trends in Pharmaceutical Research.2014;2(3): 437-458.

30. Ramadevi Bhimavarapu, Karuna Priya Chitra, Karunkiran P, Raviteja G, Meharagavendra Y, Sundaramma.S. Itraconazole loaded microsponges- A novel carrier system. International Journal of Inventions in Pharmaceutical sciences. 2015; 3(1): 953-957.

31. Mahanjan Aniruddha G, Jagtap Leena S, Chaudhari Atul L, Swami Sima P, Mali Prabha R. Formulation and evaluation of microsponge drug delivery system using Indomethacin. International Research Journal of Pharmacy. 2011; 2(10):64-69.

32. Ravi R, Senthil Kumar SK, Parthiban S . Formulation and evaluation of the microsponges gel for an anti acne agent for the treatment of acne. Indian Journal of Pharmaceutical Science and Research. 2013; 3(1): 32-38.

33. Mohan Kumar V, Veena N M, Manjula BP. Formulation and evaluation of microsponges for topical drug delivery of Mupirocin. International Journal of Pharm Tech Research. 2013;5(3): 1434-1440.

34. Swetha A, Gopal Rao M, Venkata Ramana K, Niyaz Basha B and Koti Reddy V. Formulation and in- vitro evaluation of Etodolac entrapped in microsponge based drug delivery system. International Journal of Pharmacy. 2011 ; 1(2) : 73-90.

35. Yadav P and Nanda S. Development and evaluation of some microsponge loaded medicated topical formulations of acyclovir. International Journal of Pharmaceutical Sciences and Research . 2014;5(4): 1395-1410.

36. Atmaram P Pawar, Aditya P Gholap , Ashwin B Kuchekar, C Bothiraja and Ashwin J Mali . Formulation and Evaluation of Optimized Oxybenzone Microsponge Gel for Topical Delivery. Hindawi Publishing Corporation Journal of Drug Delivery. 2015: 1-9.

37. Chainesh Shah, Dhiren Shah. Design and optimization of fluconazole microsponges Containing ethyl cellulose for topical delivery system using Quality by design approach. An International Journal of Pharmaceutical Science 2014;5(3): 95-133.

38. Jaya Raja Kumar, Selvadurai Muralidharan and Sanggetha Ramasamy.Microsponges Enriched Gel (MEGs): A Novel Strategy for Opthalmic Drug Delivery System Containing Ketotifen. International Journal of Pharmaceutical Science and Research. 2013;5(4): 97 – 102.

39. Roaa Nief and Ahmed Hussein. Preparation and Evaluation of Meloxicam Microsponges as Transdermal Delivery System .Iraqi Journal of Pharmaceutical Sciences.2014; 23(3) :63-74.

40. Ahmed Abbas Hussein. Preparation and Evaluation of Oral Microsponge Drug Delivery System of Ketoconazole . American Journal of Pharmaceutical Sciences. 2014; 14(1) :1-8.

41. Kirti A Londhe, Sheetal B Gondkar, Ravindranath B Saudagar. Preparation and Characterization of Diclofenac Sodium Loaded Microsponges for Capsules. American ournal of PharmTech and Research. 2014; 4(6): 408-413.

42. Ravi R and Senthil Kumar SK. Standardization Of Process Parameters Involved Erythromycin Microsponges By Quasi Emulsion Solvent Diffusion Method . International Journal of Pharmaceutical Development & Technology. 2013;3 (1) : 28-34.

43. Prashant Walke, Prashant Khairnar, Narkhede MR, Nehete JY .Solubility Enhancement Of Diacerein By Mannitol Solid Dispersons . International Journal of Pharmacy and Pharmaceutical Sciences . 2011;3(4): 261-264.

44. Nitin Maski, Arulkumaran, Kundlik Girhepunje, Prashant Ghode , Surajkumar Randive and Ranju Pal. Studies On The Preparation, Characterization and Solubility Of B-Cyclodextrin –Diacerein Inclusion Complexes. International Journal of Pharmacy and Pharmaceutical Sciences. 2009 ;1(3): 121-135.

45. Patel Krushika Sendhabhai and Sunita Chaudhary. Formulation and Evaluation Of Bioadhesive Buccal Tablet Of Diacerein. International Journal of Pharmaceutical Research and Bio Science. 2014; Volume 3(2): 860-879.

46. Randa Zakia, Adel Alia, Shahira F El Menshawea And Ahmed Abdel Baryb. Formulation and In -Vitro Evalution Of Diacerein Loaded Niosomes. International Journal of Pharmacy and Pharmaceutical Sciences.2011; 3(2):515-521.

47. Janki Patel, Jui Trivedi and Sunita Chaudhary. Formulation and Evalution of Diacerein Emulgel for Psoriatic Arthritis. International Journal of Pharmaceutical Research and Bio Science. 2014; 3(2): 625-38.

48. Deshmukh DB, Akwad PDG, Bankar VH and Pawar S. Dissolution enhancement of poorly water soluble Diacerein by Solid Dispersion Technique. Journal of Pharmaceutical Sciences and Research. 2010; 2(11) : 734-39.

49. Kotta Kranthi Kumar, Saikanth K, Sabareesh and Dorababu. Formulation and evaluation of Diacerein Cream. Journal of Pharmaceutical and Clinical Research. 2011;4(2): 93-98.

50. Randa M, Zakia, Adel Alia, Shahira F El Menshawea and Ahmed Abdel Baryb. Effect of Binary and Ternary Solid Dispersion prepared by Fusion method on the Dissolution of Poorly water Soluble Diacerein. International Journal of Drug Delivery.5(2013): 99-109.

51. Richa D and Sandeep VN. Development and characterization of Diacerein and chlorzoxazone Pulsatile release tablet dosage form. International Journal of Pharmacy.2015;5(2): 464-471.

52. Subramania Nair Meyyanathan. Design, Development of Diacerein sustained release matrix tablets. African Journal of Pharmacy and Pharmacology.2014;8(26):706-712.

53. Dhanashri Subhash Yadav, Ashwini Adhikrao Yadav, Poonam Subhash Karekar, Yogesh Vilas Pore and Pankaj Sadashiv Gajare. Preparation and characterization of Diacerein microcrystals. Scholars Research Library Der Pharmacia Lettre. 2012; 4 (2):428-435.

54. Veren Wally, John Lister, Lilly Desoza. Topical diacerein for epidermolysis bullosa: a randomized controlled pilot study. Orphanet Journal of Rare Diseases.2013;1-3.

55. Sohan Chitlange, Ganesh R Pawbake,Amir Mullaand Sagar Wankhede. Stability-indicating HPTLC method for estimation of Diacerein in Pharmaceutical dosage form. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2010 ;1(2):226-234.

56. Sanjay Bais, Indrajeet Singhvi, Anil Chandewar Mrunal Shirsat. Novel Colorimetric Methods for quantitative estimation of Diacerin from capsule Formulation. Asian J. Pharm. Ana. 2013; 3(2):44-47.

57. Pulla Reddy and Ram Babu. Simultaneous Determination of Glucosamine and Diacerein in Pharmaceutical Dosage Form by RP-HPLC. International Journal of Pharmacy and Pharmaceutical Research. 2015; 2 (2): 139-151.

58. Appala Raju VVSS , Janaki Pathi P, Appala Raju N. The Estimation Of Diacerein In Tablet Dosage Forms By RP-HPLC. International Journal of Inventions in Pharmaceutical Sciences. 2013;1(5): 467-469.

59. Selvam P, Mahalingam U, Sridharan D, Thenmozhi A. A Simple UV Spectrophotometric Determination of Diacerein in Pure and in Pharmaceutical

Dosage Form. Asian Journal of Biochemical and Pharmaceutical Research. 2011; 3(1) :429-434.

60. Vijayageetha, Shantha Arcot. Simultaneous Spetrometric methods in determination of Diacerein and Aceclofenac Tablets in Chemometric methods. International Research Journal of Pharmacy.2013;4(3): 211-214.

61. Narendra Kumar Nyola and Naresh Kalra. Spectrophotometric Determination Of Diacerin In Bulk And Pharmaceutical Formulation. International Journal of Pharma and Bio Sciences. 2010;1(4) : 202-207.

62. Khemchand Gupta, Indrajeet Singhvi, Sanjay Bais. UV Method for quantitative estimation of Diacerein from capsule Formulation . Asian Journal Pharmacy.2013;3(4):124-126.

63. Gurupadayya BM, Bharatheesha BM. Validated Spectrophotometric Method for the Assay of Diacerein in Bulk and Pharmaceutical Formulations .Journal of Pharmaceutical Science and Research. 2010;2 (10):638-643.

64. Kesharwani Saurabh, Kohali Dharamveer. Development and Validation of analytical methods for Simultaneous Estimation of Diacerein and Aceclofenac in Bulk and Tablets using UV-visible spectroscopy. International Journal of ChemTech Research . 2010;2(3): 1816-1822.

65. Lozada JC et al. Osteoarthritis. Available at http:// www. emedicine. medscape.com/article/330487-overview

66. http://www.emedicinehealth.com/osteoarthritis/article_em.htm

67. www.druginfosys.com

68. Indian Pharmacopoeia, Ministry of Health and Welfare Department,Ghaziabad, India. The Indian Pharmacopoeia Commission.2014;Vol 2: 1543-45

69. www.trbchemedica.com

70. www.abcam.com

71. Current Index of Medical Specialities -1169(Update 1). 2012:233

72. Raymond C, Paul Sheskey J, Majan Quinn. Hand book of Pharmaceutical Excipients. Sixth edition. 2009:525-529,262-266,564-565,756-757,404-406.

73. United States Pharmacopoeia, 30th edition NF 25-2007. The Official Compendia of Standards. 643, Pharmacopoeial forum 32(1): 141

74. Manavalan R and Ramasamy C. Physical Pharmaceutics. Vignesh publisher.2007:332

75. Indian Pharmacopoeia,Ministry of Health and Welfare department,Ghaziabad, India. The Indian Pharmacopoeia Commission.2014; Vol 2:929-930.

76. Arun Sharma, Rashmi Sareen, Varun Bhardwaj, Vineet Mehta. Topical gel incorporated with non- ionic surfactant based solid lipid microspheres of ketoprofen: Physicochemical analysis and anti- inflammatory evaluation. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(10) :199-206.

77. Suvakanda Dash, Padala Narasimha Murthy, Lilakanta Nath and Prasanta Chowdhary. Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica. Drug research.2010; 67(3): 217-23.

78. Vishal Tandon R, Anil Mahan, Hardeep Kumar. Diacerein: a new symptomatic slow acting drug for osteoarthritis.2006;8(3):173-75.

79. Gurudeep Chatwal R and Sham Anand K. Instrumental methods of Chemical Analysis. Himalaya Publishing House, Mumbai. 2014: 2.44.